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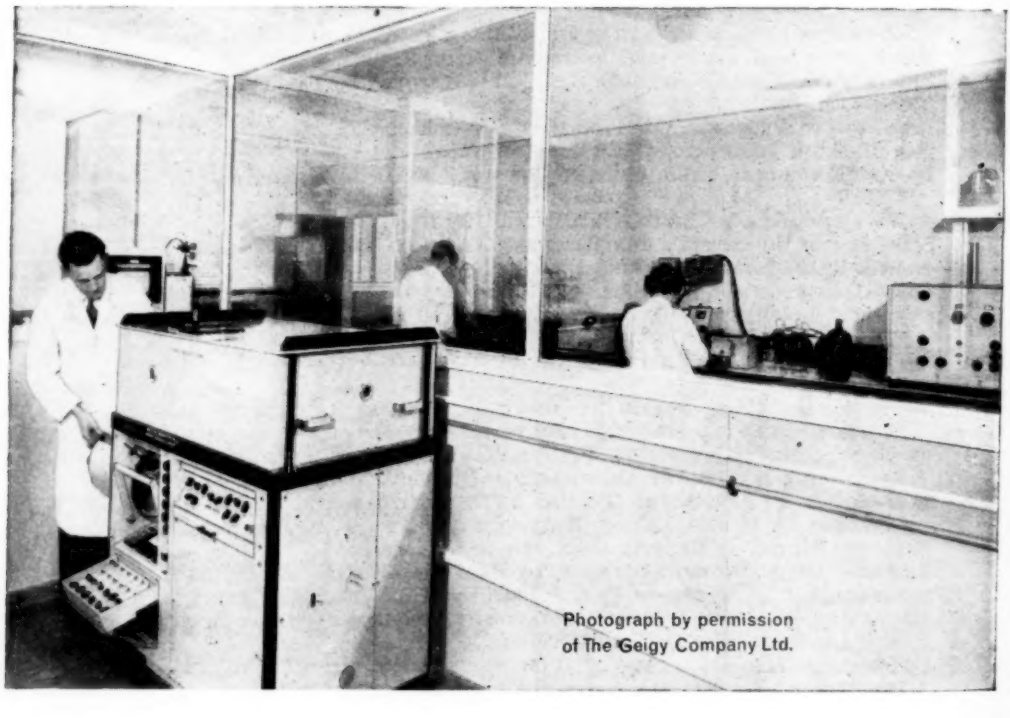

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PURPOSE IN PUBLICATION

UNTIL the middle of the seventeenth century practically all the major contributions to science had been made by men who possessed exceptional talents in other disciplines apart from scientific method. Aristotle, Bacon and da Vinci were not scientists in the way we think of them to-day: they were artists, moralists, and philosophers as well, and in those days of the non-specialist they had opportunities to pursue wide interests. To be a scientist to-day does not necessarily mean being a philosopher and artist too; but paradoxically, to be a 'good' scientist *alone* these days—a 'good' scientist being one who knows his 'literature' as well as his laboratory—is beyond the capabilities of many. This is not the fault of the scientist, nor is it due to any decline in intellectual grasp, but simply to the overwhelming mass of scientific literature which must be read to keep abreast of the subject.

It should not be deduced from this that, his scientific training and proclivities apart, the good scientist of to-day is ignorant about or, even worse, unaware of, other branches of man's culture. In fact, the contrary is true; indeed, so far as the community of scientists is concerned, the so-called 'two-cultures' (fashionably ascribed to C. P. Snow) scarcely exist. Many scientists are well read outside their own discipline, sometimes still within the ambit of science, but more often well beyond it—in philosophy, history, art, music, the theatre, literature, in fact, in the humanities generally. (Good scientists seldom make good politicians, which is probably understandable.) Some scientists of to-day have written authoritative treatises on music and musicians, and many are first-class musicians themselves; others have published poetry (in certain cases revealing that they are no mere poetasters); some have written highly successful novels; and, as used to be demonstrated at the annual meetings of the British Association for the Advancement of Science, a host of scientists are excellent pictorial artists and sculptors. T. R. Henn, lecturer in poetry and drama in the University of Cambridge, has said: "I am clear that all the great scientists that I myself have known have sought and invariably achieved some balance, some counterpoise, through a form of art, practised or enjoyed, which has taken a prominent place in their lives" (p. 537 of this issue).

In short, it is high time that the general opinion, still very extant, that the man of science is so wrapped up in his scientific literature and so confined to his laboratory that, apart from his calling, he is culturally unbalanced, be challenged. Indeed, the shoe is on the other foot; it is the student of, and savant among, the humanities, art, music and non-scientific literature (especially fiction) who are—and are often proud to admit they are—quite ignorant of science and its now 'jet-propelled' progress.

This unbalanced state of affairs in education and consequently attitudes must have contributed largely toward the movement which is fast culminating in science and technology being used for diplomatic intrigue, political propaganda, international threats and the consequent universal unrest.

It is relevant here to point out that the "Platform Party" at the opening of the magnificently posed Soviet Industrial Exhibition in Earls Court, London, on July 7 was comprised solely of Soviet and British statesmen (not including the Minister for Science) and a few others—but no scientists. Yet a very high percentage of the Exhibition was based on recent advances in science and technology. One can scarcely conceive of an exhibition of Soviet art in Britain or elsewhere being opened without the President of the Royal Academy (or his counterpart in another country) being 'officially' present.

This Exhibition clearly had a purpose other than trade. Yet, impressive though it was, it was not so revealing or informative as most trade exhibitions, and certainly not to be compared to its own advantage with, for example, the annual exhibition of the Physical Society. But advantage was obviously taken to publish a considerable amount of material, which was presumably read by many during, and after, the Exhibition. Many and varied handsomely produced brochures were made freely available. It should be borne in mind that these pamphlets will be read by some thousands of people, most of them not professional scientists, many not particularly interested in trade, and thousands of them very young and very impressionable. The background was mainly science and technology; so those responsible for the Exhibition left it to the Academy of Sciences of the U.S.S.R. to prepare some of the pamphlets. Excellently produced, some well illustrated and all issued between attractive covers, they are bound to command attention. Yet the text (in English) in each one gives a wrong impression of the *background* science which it sets out to describe. The result is that the layman will be left with the impression that nearly all the work (even back to the original) was carried out by Russians. It is true that the Exhibition was devised to show what is going on in the U.S.S.R.; but this is no excuse for confining the background, descriptive brochures to Russian work. For example, that on "Vegetative Hybridization in Plants" briefly mentions Darwin, Daniel and Burbank, then proceeds to devote the rest of its pages to Michurin and Lysenko. Of course, Vavilov is not mentioned. It is an old story, not improved by its re-telling, and significant in that it puts forward no new conceptions since the claims made by Lysenko about two decades ago. Geneticists will appreciate how misleading, therefore, this brochure is. That on "Physical Methods in Structural Analysis" states on p. 1 that: "Valuable results were achieved by G. V. Vulf, who, independently of W. L. Bragg, derived the formula under-

lying the X-ray diffraction analysis"; it then devotes nearly all the rest of its pages to present-day Soviet scientists. The brochure on "High Molecular Chemistry" adopts a similar approach.

In spite of the fact that these pamphlets were handed out at an Exhibition devoted to Soviet science and industry, it remains clear that they have been and are being read by many people, the majority of whom have scant scientific training. It can be safely assumed that few of these people will ask what contributions (original and more recent) have been made by scientists of other countries; so, to say the least of it, they will end their reading with a very distorted view of the world advancement of science.

In all these background brochures, produced under the auspices of such a distinguished scientific body as the Academy of Sciences of the U.S.S.R., very few (if any) scientists outside the U.S.S.R. are mentioned—and these only gratuitously. These leave anyone who has even an inkling of the subject in no doubt concerning the purpose of each brochure; and that is not to give a true picture of the history and present-day position of the subject. It is doubtful if many non-scientists will understand them, anyhow: they will just be overwhelmed with claims concerning Soviet achievements, and, thanks to their unbalanced education, perhaps, subconsciously, assume that nobody outside the U.S.S.R. has contributed or is contributing to the subject. The ill-informed make good material for insidious propaganda.

Coming now to the scientists in all countries, for they are not entirely blameless, they might well, with advantage to themselves and to world opinion, consider setting their own house in order. We hear much to-day about 'scientific jargon', 'scientese' and 'scientific Towers of Babel'; yet up to a point this is inescapable. The scientist must have his means of communication with his colleagues. The musician has his means of writing his scores (technically a 'language' which must be learned), and the artist also has his methods of presentation—quite often cryptic and sometimes even disturbing to the layman. It is understandable, therefore, that the non-scientist cannot comprehend much of the scientist's professional language unless the latter sets himself the difficult problem of interpreting his work in a more 'popular' fashion. (Writers of the Soviet brochures do not appear to have realized even this.)

But the 'language' of science has now got itself into such a state that sometimes even one scientist cannot understand another.

When, in 1665, the *Philosophical Transactions of the Royal Society of London* were first published, the stage was set for the scientific boom. There were 10 scientific journals in 1750, 1,000 in 1850, 10,000 in 1900, and to-day there are about 100,000. The number of journals has doubled itself every fifteen years or so since 1665 and would seem to indicate that by the year 2000 we shall have reached the million mark. Not only has the number of journals increased, but so also have the sizes of existing journals.

However, there are reasons for believing that the creation of new journals may not be as balanced a process as it might be. To take a specific example, the increase in importance of research in biochemistry, physiology and fundamental and applied biology has been very rapid during the past sixty years. (*The Biochemical Journal* was first published in 1906 and contained 495 pages; in 1926 the number of pages had risen to 1,376, and by 1960 to 2,510.) There is no reason for believing that the volume of publishable research work will decrease in the future, and one wonders whether, despite the startling rise in the number of scientific publications in general, there are enough journals to cope with the amount of material in pure and applied biology.

In the fields of mathematics, fundamental and applied chemistry and physics, and in technology, there are more journals and they are produced more frequently; as a result there usually is a shorter time-lapse between submission of the manuscript and its publication. This is not the case with the biological journals. There is none which deals exclusively with biological or biochemical research published more frequently than once a month; indeed, most are produced quarterly or even less often than that. Consequently, papers accumulate in numbers which cannot be dealt with in current issues, and in a short time there is a long waiting list.

Nature itself now has a waiting list of biochemical, physiological and biological material in spite of the fact that the journal is becoming unbalanced because it is forced to contain so much on these subjects.

Nature is a weekly journal of science and as such produces items on most scientific topics. A glance at a recent issue shows, however, that in the "Letters to the Editors" section, about 70 per cent of the contributions are on topics of pure and applied biology. Of fifty-one letters, ten are in the biochemistry section and another ten under the heading of biology. It would seem that authors, not prepared to wait for their papers to appear in full elsewhere, will submit them to *Nature* first, with the intention of writing a detailed account for a more specialized journal later. The phrase, "Further details will be published elsewhere", is becoming a familiar sight to the Editors. The phrase is justified if the communication is confined to the gist of the research work; but frequently it turns out that the paper could not contain much more detail if the author(s) tried. Moreover, complaints have been received that the indefinite word "elsewhere" is tantalizing to those sufficiently interested and who wish to examine the details; and, worse still, sometimes such "details" never see the light of day anyhow.

What is needed is either the creation of a new journal specializing in applied biology, biochemistry and physiology, to be published more frequently than the present ones, and/or for applied biologists and biochemists to use pre- and already- existing journals which have, for some reason or another, become 'unfashionable'. There is no doubt that there will be room for both these ideas as the amount of biological and biochemical research continues to increase.

The idea of a journal like *Nature* is to keep its readers informed about what is going on in *all* fields of science. Therefore, biological, physiological, and biochemical papers in particular, should be more brief and concise.

From another very serious point of view, the time-lag in publication of papers and even short communications is a blessing in disguise. It sometimes prevents authors from publishing erroneous results or unjustifiable conclusions. This, no doubt, is due not only to the enormous increase in scientific research throughout the world to-day but also to the tendency among far too many scientists to rush into print—a tendency all too prevalent and one to which the attention of the scientific world has been directed on several occasions in these columns. While waiting several weeks (sometimes even months) for publication after submission, research workers have found thrust on themselves time for reflexion, time for correction (sometimes necessary) and time for continuation of their work. It is no unusual thing nowadays for contributors to *Nature* to ask for their contributions to be withdrawn entirely or, in any event, returned to them because they have realized either errors in experimental method or in reading results or in coming to unjustifiable conclusions. Recently, on *one* day, letters for publication from three different groups of research workers were received asking for quite important changes to be made in their manuscripts already submitted, but (fortunately for them) not already published. Were it not for a certain amount of delay in publication, especially in the life sciences, the number of errata notes appearing in *Nature* would be deplorably high.

Another comparatively recent innovation, which is now becoming intense, is the establishment of so-called 'press relations' and the appointment of 'press officers'. This is, of course, not confined to science; in fact, scientific research centres are just following a general trend in this connexion. This will be all to the good for scientific advancement and information, provided it is carried out with tact, care and skill.

Nature has developed a close liaison with other journals, the newpaper Press and broadcasting organizations. Indeed, we consider this essential for both scientist and layman. Moreover, *Nature* has frequently advised scientists not to be too aloof and to do their utmost to assist the lay Press whenever their help can be of some specific value.

To-day, however, the tendency in this connexion among some scientific institutions and laboratories—academic, State and industrial—should be viewed with some concern. The number of so-called 'press hand-outs' and 'press releases' which reach *Nature* editorial office each and every day far exceeds the number of articles and communications submitted directly for publication. This might well undermine the general development and enhancement of science. For example, some press releases contain scientific results or claims that are quite fresh in that they have hitherto not been submitted to a learned journal or society for evaluation and possible criticism. Similarly, press conferences are so many and varied that they might well eventually defeat their own purpose. In any event, such 'exclusive' press releases often mean that some potential readers are missed, for it can be assumed that many such releases soon find their way into the editorial waste paper basket. The number received at *Nature* office each week would, if published, make a sizeable book. Press releases also result in duplication of publication. This may be all to the good where the lay Press is concerned (provided the material thus published in several newspapers is scientifically sound), for different people read different newspapers and magazines; but duplication in the scientific Press is highly undesirable, for undue prominence might ensue, and space is too valuable.

Nevertheless, in our own experience, press releases can sometimes be a nuisance (apart from the time-consuming job of reading them) and sometimes even dangerous. Too many of them exhibit the craze for priority or attempts to obtain the kind of publicity which is not always desirable. For example, recently a research publication was submitted to, and accepted for publication in, *Nature*. Shortly afterwards, a press release dealing with the same topic was received at *Nature* office before the original had been published. In order to keep as authoritative as possible, *Nature* very seldom publishes press releases in the form in which they are received; but on occasions they inspire the Editors to obtain an authoritative account of the subject. In the case cited, if the Editors had not noticed that the press release was a mere repetition (in different style) of what had already been submitted, double publication might have resulted in one and the same journal. The time seems ripe when editors of science journals must have a superhuman memory or possess some form of computer which will deal adequately with press releases.

Perhaps from the point of view of scientific advancement the most dangerous form of press release is that obviously written by a press officer who is not specifically acquainted with the subject under review but is doing his best to interpret some scientific finding to the lay Press. A further recent experience in *Nature* office will illustrate this. A fairly important discovery was made outside Britain by a scientist well known for his excellent work and publications. Yet a press release of his most recent discovery, presumably written by a press officer (this time in Britain), was received at *Nature* office. It was obviously meant for the lay Press, and some newspapers published it. Yet, when the Editors of *Nature* approached the scientist himself he disclaimed all knowledge of his discovery having been released to the Press.

Publication is one of the most important facets of the advancement of science. This applies not only to the scientific journal written by scientists for scientists but also to the lay journals and newspapers which are the forum for scientific exposition and discussion on the impact of science on society. In the past, far too many scientists have tended to remain aloof from the layman, and most have scorned

any form of publicity. To-day, however, scientists are becoming increasingly aware of the effect of their calling on world society; this is no doubt due mainly to the tremendous strides science (and especially its applications) have made during the past few decades. Now, it is to be hoped that the pendulum is not swinging too far the other way. It is certain that claims for priority are uppermost in too many minds, and there is far too much inter- and intra-national competition and still not enough collaboration and team-work among laboratories and even nations.

Perhaps the most objectionable activity is that of the scientist who is determined that his light shall not be hidden under a bushel and therefore acts as his own press officer. Fortunately, in our experience such men are rare; but they are with us to-day, sometimes going so far as to notify the lay Press that they are about to publish in a scientific journal a communication in which the newspapers may be interested—even before they know whether the communication to the scientific journal has been accepted. Frequently succeeding in their endeavours, such scientific activities achieve publicity out of all proportion to their importance. There may be risks that ill-based claims are read and accepted by many readers.

Perhaps the day is not far off when scientists, through their means of publication, may be divided into the tough and the tender by a future William James, or the idealists and the realists by a future Schiller, or the "extraverts" and the introverts by a future Jung. It is certain that a future editor of *Nature* will be able to distinguish between the sincere scientist and the self-centred or personally ambitious scientist; but, alas, that editor may not live long enough to satisfy himself that the former is the more valuable member of any scientific team or community.

The scientist most desirable to science and society is he who leaves the evaluation of his work to his fellow scientists, presenting his findings with that modesty and dignity which one should always be able to associate with the word 'science'.

WHAT IS BASIC SCIENCE?

Disease and the Advancement of Basic Science

Edited by Dr. Henry K. Beecher. Pp. xi+416. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1960.) 100*s*. net.

THIS collection of articles illustrates the thesis that a study of disease can advance basic science. This thesis might seem self-evident, but it has not turned out to be so. During this century, and particularly in Great Britain, to try to understand Nature's experiments, as exemplified by disease, has tended to become scientifically disreputable. Thirty years ago Sir Thomas Lewis carried out a brief campaign for the recognition of clinical science as a discipline comparable in scope and status to those of other recognized branches of biology. He quoted the words of Sir James Paget of many years before: "I feel sure that Clinical Science has as good a claim to the name and rights and self subsistence of a science as any other department of biology; and that in it are the safest and best means of increasing the knowledge of diseases and their treatment. . . . Receiving thankfully all the help that physiology or chemistry or any other sciences more advanced than our own can give us, and pursuing all our studies with the precision and circumspection that we may best learn from them, let us still hold that, within our range of study, that alone is true which is proved clinically, and that which is clinically proved needs no other evidence". Lewis's advocacy produced an adverse response from medical scientists whose allegiance was primarily to the laboratory. In his presidential address to the Royal Society in 1934, Hopkins expressed the suspicion that the fields of clinical science so brilliantly exploited by Lewis and Mellanby were probably very few. He said: "I seem to have sensed, however, the beginnings of a definite movement in this country, and indeed elsewhere, not, of course, to ignore the laboratory; but in the distribution of funds provided for medical research to endow the Clinic on a scale which might endanger the future of research in fundamental biological science. The tenor of my remarks has been due to a conviction that in the long run such a policy would sterilize advance".

No detached observer would claim that the study of disease has made contributions to technique or to detail comparable with those achieved in the laboratory. But laboratory science has one major danger: it tends to be canalized into those lines that are, for the time being, most productive; the contemporary massive concentration of physiological effort into the behaviour of excitable tissues is an example. The unique contribution made by a study of disease is the prevention of too narrow an outlook and interest. How this has been and is being done is illustrated by the twelve articles in this book. Three may be quoted.

The first is unique, in that Linus Pauling conceived the idea that there might be molecular disease, that is to say, a disease the features of which were the direct outcome of the composition of a specific molecule. From what he was told of it, he wondered if sickle-celled anæmia could be due to a different form of hæmoglobin that crystallized out in the reduced form. This he was able to demonstrate. This story has recently culminated in Ingram's demonstration that normal and sickle-cell hæmoglobins differ by the presence of two amino-acid residues of valine in hæmoglobin *S* corresponding to amino-acid residues of glutamic acid in hæmoglobin *A*.

Bearn relates the story of biochemical genetics: how the story really began with Archibald Garrod, with his studies on the "Inborn Errors of Metabolism", albinism, alkaptonuria, cystinuria and porphyria. Garrod's work led him to consider that the abnormal gene resulted in specific biochemical abnormality which, in turn, gave rise to disordered metabolism and disease. This idea was revived, sharpened and more widely illuminated by the brilliant work of Beadle and Tatum on the eye colour of *Drosophila* and the nutritional requirements of *Neurospora* resulting in the one-gene/one-enzyme hypothesis. Bearn goes on to discuss the remarkable advances in our knowledge of copper metabolism produced by study of Wilson's disease, hepatolenticular degeneration, a disease transmitted by a Mendelian recessive gene and characterized by an inability to synthesize the copper-containing protein of serum.

which, because of its colour, has been called ceruloplasmin. This is one of the more recent examples of how a genetically transmitted disease can illuminate biochemical mechanisms.

Perhaps the most enthralling story told in these pages is that of pernicious anæmia, first described by Addison in 1855. The break-through in this disease occurred when in 1926 Minot and Murphy showed that a diet of half a pound of liver a day would cure the disease, using as their index of response the percentage of immature cells, or reticulocytes, among the red cells of the blood. Minot and Murphy's trial of liver was based on a false analogy with experiments by Whipple, in which he had shown that liver was one of the most potent dietetic factors stimulating blood regeneration after bleeding in the dog. The analogy was false because the efficacy of liver in the dog was later shown to be due to its iron content, while in man post-hæmorrhagic anæmia has not been shown to respond to liver. There was an old series of observations suggesting that the primary defect in pernicious anæmia lay in the stomach, for Handfield Jones in 1854 had described atrophy of the glandular portion of the stomach in what was probably pernicious anæmia, and in 1870 Fenwick had shown that scrapings of the gastric mucosa, to which diluted hydrochloric acid had been added, failed to diminish the weight of a cube of egg white on incubation. The stomach defect had also been shown to precede the onset of anæmia. These two series of observations were brilliantly synthesized by Castle, who showed that if a normal subject was fed one pound of lean beef and made to vomit it at the end of an hour, and if the vomit was then fed to a patient with pernicious anæmia (as Castle remarked, *honi soit qui mal y pense*), the patient responded with a reticulocyte response and cure of the anæmia. Evidently, two factors were involved in the genesis of pernicious anæmia, an extrinsic factor present in lean beef, and an instrinsic factor present in the normal human stomach but absent from that in pernicious anæmia. The story is now nearly complete. The extrinsic factor is known to be the complex molecule of vitamin B_{12}, cyanocobalamin, the structure of which was so brilliantly identified by Todd and Hodgkin. The intrinsic factor still awaits precise definition, but there is no doubt that it is produced by the stomach and that its function is to facilitate the absorption of vitamin B_{12}.

These essays prompt the question: What is meant by basic or fundamental research? Listening to applicants for research grants, I would have very little doubt that the two adjectives have a substantial snob, prestige or prig value, as you care to look at it. All scientific workers wish to claim that what they do is basic or fundamental. The terms have also been applied to laboratory work as opposed to work outside it. Another usage equates basic science with pure science, the rest is applied. I have to confess that the intention to divide the pursuit of knowledge into fundamental and applied, into useless and useful, into respectable and unrespectable, has always been, and is, utterly repugnant to me. Knowledge is a unity, and an experiment devised and carried out in the laboratory is not necessarily more interesting or more illuminating than one designed, as it were, by the hand of God. It is at once conceded that it is often more difficult to apply the experimental method to the study of disease in man, and that to unravel the experiments of Nature is more difficult than to unravel those designed by man. But the present tendency to laud the laboratory and decry field work of whatever kind is not in the best interests of scientific knowledge as a whole. As Roger Bacon wrote, "All sciences are connected; they lend each other material aid as parts of one great whole. . . . As an eye torn out or a foot cut off, as it is with the different departments of knowledge; none can attain its proper result separately, since all are part of one and the same complete wisdom".

GEORGE PICKERING

THE OCEAN FLOOR

The Floors of the Oceans

1: The North Atlantic. Text to Accompany the Physiographic Diagram of the North Atlantic. By Bruce C. Heezen, Marie Tharp, and Maurice Ewing. (Geological Society of America Special Paper 65.) Pp. xii+122+30 plates. (New York: Geological Society of America, 1959.) 4.50 dollars. Physiographic Diagram 1.50 dollars.

UNTIL very recently very little was known of the geology of the oceans, and rapid growth of interest and improvement of methods and instruments during the past fifteen years have led to striking advances. Improved echo-sounders, deep-sea corers, underwater cameras, towed magnetometers, shipborne gravimeters and other techniques have disclosed a new field for scientific exploration and geologists are rapidly taking advantage of it. Expanding interest in the history of the oceans, the nature and structure of the sediments and igneous rocks on the sea floor, and the processes at work, was one of the most striking features of the first International Oceanographic Congress held two years ago. The new idea of a borehole in the ocean floor was mentioned, and already an American team operating through water two miles deep has taken continuous samples from a borehole through 500 ft. of sediment and some 50 ft. of underlying rock.

The Lamont Geological Observatory, founded within Columbia University in 1949, has been outstanding in pioneering underwater geology, and this account of the floor of the North Atlantic Ocean by Heezen, Tharp and Ewing is based largely on first-hand experience. They show how they have been able to produce a "Physiographic Diagram of the North Atlantic Ocean" and outline its usefulness. The diagram is published separately for wall mounting, and it is the first of a series planned to cover the oceans of the world. It gives a striking portrayal of the general topography and roughness of the ocean floor. The horizontal scale is about 1 to 5 million, and the vertical scale has to be exaggerated by about 20 to 1 to bring out relief. It might be argued that this exaggeration over-emphasizes the slopes and the sharpness of features, but it is probably about the best compromise. There are nothing like enough soundings to base all the detail on fact, but the interpolation and extrapolation undoubtedly give as realistic a picture as we are likely to get for a long time.

The authors make some systematic classification by dividing the topographical features into three main types: continental margin, ocean-basin floor, and mid-ocean ridge, and subdividing these, sometimes tentatively, into component elements. The top part of the mid-ocean ridge, for example, is divided into rift valley, rift mountains, and high fractured plateau.

XUM

The distinctions they make are illustrated with the help of sounding records, bottom profiles, charts and underwater photographs, and, where possible, with the results of coring and dredging, magnetic and gravity anomalies, seismic soundings and heat-flow measurements.

Among the most remarkable features are the abyssal plains, where the underlying topography has been smoothed over by sediment-laden currents so that only the highest hills and seamounts stick out like islands from the surface of a lake. Mid-ocean canyons are steep-walled, flat-floored channels, 1–5 miles wide and perhaps 100 fathoms deep, cut in abyssal plains, generally in the approaches to steps to neighbouring, lower, plains. The mid-ocean ridge is about 1,200 miles wide with varied and irregular topography, and having a median rift, the rift valley, which, so far as we can see, is continuous down the length of the ridge.

Their new ideas are presented carefully, and, while giving much new information and many new ideas, the authors show where they need further support, and what we ought to do next. The book is a special paper of the Geological Society of America. Like several of the Society's publications, it will do much to further the study of the sea. It should be useful to cable companies, because of the new light it throws on some of the natural hazards and destructive processes that occur on the sea-floor. It has an excellent bibliography.

G. E. R. Deacon

SECTOR-FOCUSED CYCLOTRONS

Sector-Focused Cyclotrons

Proceedings of an Informal Conference, Sea Island, Georgia, February 2–4, 1959. Edited by F. T. Howard. (Nuclear Science Series—Report No. 26. Publication No. 656.) Pp. xii + 291. (Washington, D.C.: National Academy of Sciences—National Research Council, 1959.) 2.50 dollars.

ACCELERATOR development has progressed with two main objectives in view: the first, to generate higher and higher energies, and the second, to obtain greater intensities. It was in the interests of this latter aim that the Sea Island Conference was held. Scientists from laboratories in America and Europe met to exchange information and ideas on the ways of increasing the intensities of medium- and high-energy cyclotrons by employing sector-focusing. It was fitting that L. H. Thomas, the first to expound this principle in 1938, should be among those present. Having been interested meanwhile in other subjects he could be forgiven his surprise at the delayed-action effect of his work.

The application of frequency modulation to cyclotrons in 1946 effectively solved the problem of obtaining higher energies but only at the expense of intensity, the continuous beam of the conventional cyclotron being sacrificed for a pulsed beam having a duty cycle unattractive for many nuclear experiments. For a time the attractions of the higher energies quenched the desire for greater intensity until of recent years its pursuit has been revived in several laboratories.

The *Proceedings* of the Conference record the papers and discussions at six sessions, the first of which was devoted to theoretical studies and orbit calculations. In this work, the use of large computers had proved invaluable. The achieving of the required magnetic field, however, was generally by way of successive approximations on an experiment model initially and later at full scale. Various methods of magnetic field measurement were described, many of which were mechanized to reduce the amount of repetitive work.

In the session devoted to radio-frequency problems novel approaches had been made in order to obtain a wide tuning range to give variable energy facilities. Then followed a session on quality of the beam, when interesting results were reported from several laboratories. This led naturally to problems of beam-extraction in which resonance phenomena, either deliberately induced or inherent in ridge-focusing, were discussed. The final session included a variety of topics relevant to experiments on cyclotrons, not the least important of which was that of shielding.

Approximately fifty papers were presented and the attendance of more than 80 is indicative of the wide interest in this field of work. Since the Conference several of the accelerators mentioned have come into operation. Perhaps these successes were in some measure helped forward by useful information exchanged there and certainly future workers will benefit from its recording in this series.

M. Snowden

THE PROBLEM OF RETIREMENT

Growing Old in a Mechanized World

The Human Problem of a Technical Revolution. By F. Le Gros Clark. (Studies of Ageing within the Conditions of Modern Industry.) Pp. 147. (London: The Nuffield Foundation, 1960.) 7*s*. 6*d*. net.

MR. F. Le GROS CLARK, with the same clear insight into the urgent practical problems confronting the older worker, restates his theme and provides us with additional data. What is happening to and what is likely to happen to the working man between the ages of 60 and 75, if he remains in good general health, wishes to continue in employment, but because of declining powers finds it more and more difficult to hold his own in the conditions of modern industry? What effect is the progressive trend towards mechanization and automation likely to have on the prospects of the ageing man? To provide some data towards an answer to these questions, Mr. Le Gros Clark takes eight technologically advanced industries (motor-cars, rayon yarn, plate-glass, etc.) and examines the position of the older worker in sample factories.

His conclusions are fairly pessimistic—more pessimistic than those of his earlier reports, also published by the Nuffield Foundation. The accelerating progress of mechanization and automation is beginning to affect the prospects of the older employee. Not only is automatized work more demanding in itself (in the need for vigilance, paced performance, etc.) but also the range of alternative 'light' jobs to which older workers might have been transferred is becoming progressively limited. A rigidly imposed retiring age is making it increasingly difficult for the fit man of 65 or more to remain in employment. We must expect to find more and more men retired from manufacturing industry while they are still reasonably healthy. Are these men likely to find alternative work, if they wish to, in other fields of employment? In a long appendix Mr. Le Gros Clark considers the older man's prospects in other sorts of work

road maintenance, gardening, public services, caretaking) and comes to the conclusion that most of these jobs cannot provide alternative employment for many manual workers who apply well beyond their middle lives.

So what can be done ? Mr. Le Gros Clark has two main suggestions : That we should call into existence new categories of light work (he mentions 'paid social work' but does not define what he means by this) : and that we should work out a new social theory of retirement. In other words Mr. Le Gros Clark is now (reluctantly ?) coming to the conclusion that the period of retirement is "the inescapable third period of life" even for those who are fit and well. "We have spent much of our time studying what chances we have of preserving the health of older men and of adapting factory conditions to their changing physique. We have spent even more of our time studying the needs of the infirm and aged. It is now our business to add to these a third and equally important field of research—that into the full meaning and content of retired leisure". This may well mean a re-casting of social values.

L. S. HEARNSHAW

DALTON'S IRISH RIVAL

The Life and Work of William Higgins, Chemist (1763–1825)

Including reprints of "A Comparative View of the Phlogistic and Antiphlogistic Theories" and "Observations on the Atomic Theory and Electrical Phenomena" by William Higgins. By Prof. T. S. Wheeler and Prof. J. R. Partington. Pp. viii + 173 + xiv + 496. (London and New York: Pergamon Press, 1960.) 60s. net.

THE mere sight of the title of this work will give historians of chemistry (and especially of atomism) great satisfaction. The biographical and critical matter which makes up the modern third of the volume could probably only have been produced by this collaboration of these particular authors. The publishers are to be congratulated on reprinting in facsimile the works of William Higgins, which are discussed in every history of atomism but can in fact have been seen by only few readers. It is an appropriate tribute to one of the many Irishmen who did so much to advance science in the eighteenth century.

The history of the early chemical atomic theory is not synonymous with the work of John Dalton, although he holds the centre of the stage. It seems to us now as if some re-statement of qualitative atomism in quantitative terms were inevitable at the close of the eighteenth century. However, this is historical wisdom after the event. We are not yet certain how Dalton's mind and those of his contemporaries worked. Did Higgins anticipate Dalton ? Did he influence Dalton ? Davy was prepared to say that Higgins's work had been important, but his authority has little value. False notions about the historical origins of their beliefs have often been held, and are often held now, by leading authorities in experimental science. The work which brings a man scientific eminence does not necessarily make him a reliable historian, even of his own field.

The controversies in which Higgins's name was prominent began soon after Dalton's theory was put forward, which was, however, long after Higgins's first book had been published and passed over. Higgins's *Comparative View of the Phlogistic and Antiphlogistic Theories* was printed in 1789 in London. It was a detailed refutation of Kirwan's *Essay on Phlogiston* of 1787 and is remarkable in being "the first English work to support Lavoisier".

At that time the phlogistonist controversy seemed overridingly important. Twenty years later, the newer system expressed in the theoretical language of Lavoisier was supreme. Higgins's advocacy was no longer relevant and the aspect of the book which became, and remains, interesting is Higgins's use of an atomic theory in expounding his antiphlogistic views. There is no doubt that he had a notable insight into the quantitative basis of chemistry, had grasped Lavoisier's teaching well and showed some originality in combining it with atomic ideas.

In some ways he was in advance of his time (as in his views on reaction mechanisms) and might have helped to advance chemical theory if he had possessed two qualities which in fact he lacked. One was the capacity to see wherein lay the fundamental novelty of a quantitative atomic system. The other was a capacity to express himself with clarity and self-evident consistency. Dalton possessed both these qualities, and although he may have failed to see all the consequences of his own theory, he certainly opened the eyes of his contemporaries quickly and effectively.

Dalton was recognized and honoured: in 1814 Higgins felt impelled to advance his own claims to priority by publishing his *Experiments and Observations on the Atomic Theory* reproduced in facsimile in this same volume. It could not give Higgins the high place he claimed, and which Wheeler and Partington conclude he could not justly claim. "Higgins has an honourable place in chemistry, but he does not stand on the same level as Dalton."

The notes, appendixes, and two sets of correlations between *Comparative View of the Phlogistic and Antiphlogistic Theories* and critical text are models of scholarly thoroughness. The biographical text is the first life of Higgins of any completeness.

This is not a biography for the beginner, who would in fact find much of it aridly dull. The authors have evidently not thought it necessary to extend their material by much connecting narrative or exposition intended to make its digestion easier. As an aid to study and as a record of ascertained fact, however, its faults are few.

It is not clear why the facsimile of the *Comparative View of the Phlogistic and Antiphlogistic Theories* is of the second edition, dated, so far as can be deciphered, 1791, when the earlier 1789 edition could have been used. So far as the text goes, however, this is unimportant, because comparison of facsimile with the first edition shows that the two are identical, errata and all. The errata have been corrected by hand in the original copy, but not in the original *Atomic Theory* copy. This is all of no real significance, but it is regrettable that, in my specimen at any rate, the *Comparative View of the Phlogistic and Antiphlogistic Theories* has lost the first page of the list of contents. The student will have to fill this small deficiency as best he may.

For the historian, yet to appear, who will write a worthy history of the early atomists, and will re-interpret them to a generation which consistently misrepresents them, this book will be an indispensable tool.

FRANK GREENAWAY

SCIENCE AND POETRY*

By T. R. HENN, C.B.E.

President, St. Catharine's College, Cambridge, and University Lecturer in Poetry and Drama

I

THE attack on poetry, not by the scientist but rather by the man of common sense, is nothing new in history. Plato (who was, as Sir Philip Sidney pointed out, the most poetical of philosophers, teaching by means of the very myths and parables that his dialectic drove him to condemn) had banished the poets from his ideal "Republic". Some of his leading indictments are still current to-day; in our popular press, in the mouth of the common man, and in those jarring sects and faculties that compose a university. Poets are liars; *they say the thing which is not*. They encourage their listeners to give way to undesirable and womanish emotions, which should be repressed or governed by the intelligence. They tell lies about the gods. They give us examples of men and women doing what they should not; and thus cause others to commit, or at least to contemplate, the practice of evil things.

Now this general indictment is repeated, with differing variations, stresses and tones, from the beginnings of literature; most forcibly, perhaps, by the Tudor Puritans, who found, no doubt, some social justification in the contemporary ballads, and in the condition of the theatres as breeding places of both sin and plague. We may note the heavy cross-fire, from a slightly different angle, in Peacock's attack not only on his fellow-writers of the early nineteenth century but also on the general status and office of the poet. Whatever the function of poetry in the past ages may have been, he says, it was wholly out of place in his own "Iron Age of Industrialism". It is worth quoting a passage:

"The descriptive poetry of the present age has been called by its cultivators a return to nature. Nothing is more impertinent than this pretension. Poetry cannot travel out of the regions of its birth, the uncultivated lands of semi-civilized man. Mr. Wordsworth, the great leader of the returners to nature, cannot describe a scene under his own eyes without putting into it the shadow of a Danish boy or the living ghost of Lucy Grey, or some similar fantastical parturition of the moods of his own mind. . . .

"In the origin and perfection of poetry, all the associations of life were composed of poetical materials. With us it is decidedly the reverse. We know too that there are no Dryads in Hyde Park, nor Naiads in the Regent's Canal. But barbaric manners and supernatural interventions are necessary to poetry. Either in the scene, or in the time, or in both, it must be remote from our ordinary perceptions. While the historian and the philosopher are advancing in, and accelerating, the progress of knowledge, the poet is wallowing in the rubbish of departed ignorance, and raking up the ashes of dead savages to find gew-gews and rattles for the grown babies of the age. . . .

"A poet in our times is a semi-barbarian in a civilized community. He lives in the days that are past. His ideas, thoughts, feelings, associations, are all with barbarous manners, obsolete customs, and exploded superstitions. The march of his intellect is like that of a crab, backward. The brighter the light diffused around him by the progress of reason, the thicker is the darkness of antiquated barbarism in which he buries himself like a mole, to throw up the barren hillocks of his Cimmerian labours. The philosophic mental tranquillity which looks round with an equal eye on all external things, collects a store of ideas, discriminates their relative value, assigns to all their proper place . . . is diametrically the reverse of that frame of mind which poetry inspires, or from which poetry can emanate".

II

I have quoted Peacock at some length because, with slight changes of emphasis and tone, it represents a considerable body of opinion to-day over the science-poetry controversy. We have swept away the myths, superstitions, "old furniture of the mind", the "Hebrew old clothes" on which are founded the "Magical View of Life" (the phrase is from I. A. Richards)[1] that once provided not only the materials but the very life-blood of poetry. It was a celebrated critic of a hundred years ago who advised Matthew Arnold to "leave the exhausted past". Science is the living example of reason in all things. It brings light, if not sweetness, to mankind. The history of science is that of the march of the intellect, a series of slow triumphs over ecclesiastical or political obscurantism, superstition and human stupidity. Science alone deals with *fact*, "susceptible, at every stage, of verification"; and exploiting, clearly and unequivocally, its practical 'goods'. It leads to the practical 'goods' of an ever-mounting standard of living, of medical care, of comfort, of speed, safety. It conduces steadily towards lighter and shorter hours of work. It gives man power over all things material. To such beliefs we are also inexorably driven, every day of our lives, by the assertions of commercial interests of every conceivable kind, whether overt or subliminal; and these assertions are not disinterested.

This is a common picture of a common belief: and at this stage of our discussion I wish to do no more than interject a quotation from Sir Charles Sherrington:

"It was Joseph Priestley, writing . . . twenty years before the French Revolution, who foresaw issuing from Science a future 'glorious and paradisiacal beyond our imagination'. 'Nature . . . will be more at our command: men will make their situation in the world abundantly more easy and comfortable; they will probably prolong their existence in it, and will daily grow more happy'. The prediction was correct, save for its concluding sequitur, which as we hear it now rings as a non-sequitur. Priestley's enthusiasm had thought greater knowledge must mean supremacy of 'the values'. It might but it did not. In all this man can look for no help from any mind on his planet but his own. . . . He is the only recognizer of 'values' ".

III

Let us take the present disrepute of what Sidney called 'poor poetry' a little further. The artist or poet has, since the late eighteenth century, tended to appear to the public as a lonely, perhaps eccentric figure. Theseus's implied equation of him with lunatic and the lover dies hard in the mind of the common reader. He remains separate, in a real or imagined attempt to assert his own personality, his sense of his self-importance. He often sees himself

* Substance of a lecture delivered at Hatfield Technical College on May 12.

as the one against the many. A suggestion of the *vates*, the prophet, the seer, has clung to him from the beginnings of history, and he is reluctant to jettison it. He has been in the past the inspired, even the afflicted (and therefore the venerated) of God. Many of Peacock's gibes still hold in popular estimation. If his audience believe neither in prophecy, nor in the world of the supranatural, nor in the existence of Spirit, his claim to attention may well be a thin one. As reverence for him diminishes, he is apt to clutch, even more tightly, the cloak of his real or assumed personality. The unpicturesque disorder of the 'beatnik' poet in Los Angeles or Greenwich Village is as much a badge of office as the long hair, the flowing tie, of the 'decadent', whether alcoholic or homosexual, of the 'nineties. There is more than a suspicion that his morality, particularly in sex, drink and drugs, will never stand the scrutiny of biographers, or even of the courts: the lives of the poets can be as unedifying to-day as they were, on occasion, to Johnson or Arnold.

With these things go a traditional inability or unwillingness to engage with any notable success in the world of action. We remember that Aeschylus recorded on his tombstone the fact that he had fought at Marathon, and left no word of his dramas. Sophocles commanded a squadron, and:

> "Got laughed at, and went back to making plays,
> Having allowed us our experiment
> Respecting the fit use of faculty".

Chaucer seems to have been an efficient Civil servant and diplomat; Spenser did his job as what we should now call a military assistant to Lord Grey on his Irish Expedition. Marvell was a tolerable member of Parliament for Hull; Milton became First Secretary to the Commonwealth because he was the first Latinist in Europe. Byron had a military-political career that was both dramatically satisfying and reasonably efficient. But for the rest it is a depressing record of temporary and rather futile incursions into the active life—Gibbon, Coleridge, Wordsworth, Shelley, Landor are witnesses. In our time, in this century, I know of no major poet (with the possible exception of Wilfred Owen, who was killed at the crossing of the Sambre Canal on November 4, 1918, and of Sidney Keys in the Second World War) who was engaged in action; though I have known two generals who were minor poets themselves, and we may recall Roy Campbell, Brooks, Masefield and a few others. It is little wonder that our common man is suspicious of the poet's claims to supreme importance, to be the potential saviour of civilization, even to be the provider of a substitute for religion. It is very clear to the common man that poets are not, and never have been, Shelley's "unacknowledged legislators of the world". He can make no sense of Wordsworth's claim that "poetry is the breath and finer spirit of all knowledge". He observes with uneasiness that the poets, so far from facing up to their own particular problems, seem to be talking to their own small circle in a language of unexampled and ever-increasing complexity; and when, with a sense of relief and even pleasure, he turns to recognize his own sensible nostalgia in the work of Mr. Betjeman, he is told by the experts that this is not poetry. In the present literary scene the common man or scientist will note that only two literary figures of importance have taken degrees in science or mathematics, and he will reflect sadly that one has returned from defending poetry to a divided loyalty between the Coal Board and the B.B.C., and that the other is apt to write poetry which is comprehensible with difficulty even to the mathematicians themselves. (Curiously enough, there seems to be some tendency for biologists to be artists—perhaps because of much study of illustrative drawings—and for mathematicians and physicists to be musicians. Perhaps there is something of psychological interest here.)

IV

Let us start again. Very few poets have attempted any kind of 'liaison of vocabulary' between science and poetry. It is true that when science was younger it could and did lend to poetry material for metaphors; some elementary geometry for Marvell; optics, metallurgy, chemistry, a few medical terms, to John Donne; much alchemy, a little astronomy, and a vast quantity of astrology. It is, I think, true to say that no poet to-day would dare to take sides in verse in the Ryle–Hoyle controversy over the origin of the universe. "Never write a poem about the solar system" is a sound maxim; though I notice that this year at Cambridge the prescribed topic, "for a poem conducive to the honour of the Supreme Being and the recommendation of virtue", is "The Spacious Firmament". (But it is only fair to the University authorities to say that this was prescribed before the appearance of the space man.) There is a fair amount of contemporary science reflected in Shelley and Coleridge (in the main, chemistry and physics) and in Tennyson, who is strongly affected by Darwinism. The attempts to find, at the heart of the nucleus, an "Element of Purpose", suggesting the existence of creative mind, do not appear to me to have been very successful; nor is there (as Schroedinger has pointed out) any ascertainable relationship between the quantum theory and the problem of free will. It is true that certain possibilities of science in our time have stirred momentarily the poetic imagination. So D. H. Lawrence's *Relativity:*

> "I like relativity and quantum theories
> because I don't understand them
> and they make me feel as if space shifted about
> like a swan that can't settle,
> refusing to sit still and be measured;
> and as if the atom were an impulsive thing
> always changing its mind."

or the attempt to find free-will restored by Einstein:

> "But to one muddled mind it seems
> A curvilinear universe
> Involves free-will, and so redeems
> God and the world from Calvin's curse."

But on the whole it seems as if modern poets distrust technological achievements; as in William Empson's poem "To an Old Lady" (which is the Moon):

> "Project her no projectile, plan nor man it . . .
> Landing, you break some palace and seem odd . . .
> No, to your telescope, spy out the land:
> Watch, while your ritual is still to see."

The poets seem, on the whole, to look backward with some longing to the period before the break-up of Hellenic and Hebrew mythological knowledge; consider Ronald Bottrall's *Eppur Si Muove:*

> "there must be horse-sense, horse-strength, to spare
> For building up into new stars
> The molecules that the great machine
> Has broken up into minute particulars
> Remote from all that's viable and keen."

XUM

It will not work; any more than did Erasmus Darwin's *Botanic Garden*, or the *Loves of the Plants*. And although I have sometimes thought that some aspects of electronics might lend themselves to images in love-poetry—we might, for example, ponder on the possibilities of 'transistorized' and 'superheterodyne', or even of the experience of love as a 'reactor' rather than the classic 'crucible' or 'alembic'—it is clear that the 'multitudinous twittering polysyllables' of the average scientific paper do not lend themselves readily to rhythmic or musical utterance. Let us abandon all thought of these pseudo-marriages between the two sides, and start afresh with our argument. Poetry and science are separate, as human activities; and here I want to quote from Matthew Arnold:

> "It is the task of science to ascertain the facts of life; and the task of poetry to relate them to human experience".

But what are the "facts of life" to-day; and how does poetry claim to relate them "to human experience"?

V

Let me attempt to continue my case by postulating a scientist of high intelligence, who has studied (and on the whole assents to) the familiar case for the denigration of poetry, or at least the relegation of it—following Freud—to an auxiliary but peripheral activity. I should, I think, argue with him in some such terms as these.

In poetry we are concerned with statements of peculiar variety, strength and subtlety; and the operation of these statements we cannot pretend to understand without some agreement as to the nature and operation of the human mind; and in particular to the proposition that, whatever our claims to act under the stimulus of reason alone, the Emotional or *E* component, whether drawn from the conscious or subconscious, is of great importance. (There are, incidentally, grounds for believing that, for various reasons—which include those of the rapidity of modern communications—this *E* component is increasing rather than diminishing in the human situation.) Our thoughts and actions are conditioned and governed by a peculiar and varying blend of the *R*, or rational, and *E* components; and it is clear that, whether we "follow Priapus or Paul", Freud or Jung or Marx or Sartre, the single most important determinant factor is the past: whether in heredity, environment or upbringing. And this past is in itself a factor of incredible complexity. It is, inevitably, beyond and outside any accurate analysis or assessment by ourselves. It can only be reckoned with by a technique which makes use of a comparable delicacy and complexity in dealing with its effect. I am now hinting at a profound and difficult statement by Shelley, to which we shall return later:

> "Poetry administers to the *effect* by acting upon the cause."

Poetry is making statements about the nature of experience; experience that the poet perceives, organizes, refracts, and casts into moulds of the kind appropriate to his art. It is an art that is both more difficult and perhaps more satisfying than that of the painter, or sculptor, or musician; largely because the poet is using language as a multiple instrument. The formal technique of poetry must be recognized, studied, and, in a particular sense 'understood', if we are to receive what it has to communicate. The poet is making words perform peculiar yet precise functions; setting to work a complex selector-mechanism which can sometimes be shown analogically (as I have tried to do in a little book called *The Apple and the Spectroscope*) in mathematical and electrical terms; and if I were re-writing it, I should, I think, want to attempt to extend my illustrations into elementary chemistry as well. The selection, interaction and continuous modification of the meaning of words is made possible by setting them in complicated matrices of rhythm, tone, accent, pitch; themselves part of a relationship in terms of music which still further defines, amplifies and extends the meaning which the poet attempts to communicate. Further, he can and does use the method of perceiving correspondences between experiences (of events, people and objects) to indicate similarity and/or dissimilarity in that which he perceives, and hence can indicate judgments as regards value—whether positive or negative—regarding his perceptions. We may again refer to Shelley:

> "Reason is the enumeration of qualities already known; imagination is the perception of the value of those qualities, both separately and as a whole. . . . Reason respects the differences, and imagination the similitudes between things."

But what is this poetic statement about? How can we explain its significance and what is the use of it, anyway? What relevance has it to our problem of "Science and Poetry" to-day?

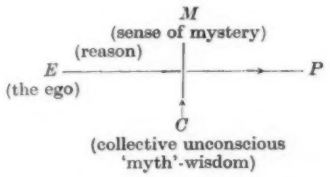
To some of these questions we can give brief but provisional answers. Poetry is 'about' the human situation, in its broadest terms. It is 'about' the highly complicated amalgam of intellect and emotion, of head and heart, of conscious and unconscious habits (but habits which grow and change continuously) that govern our normal lives. To the amalgam of the *R* and *E* components we are committed, inescapably, by the human situation. From the beginnings men have attempted to hack through the barrier, to behave in marriage, politics, war, as if humanity were (or might, one day, be) wholly 'rational' in its operations, as in the cold world of Swift's horses. It was in relation to love and marriage and woman's intuition that Meredith complained so bitterly:

> "Their sense is with their senses all mixed in;
> Destroyed with subtleties these women are.
> More brain, O Lord, more brain, or we shall mar
> Utterly this fair garden we might win. . . ."

But it has not happened like that, and it will not: public and private history, religion, love, philosophy, all proclaim through time the irrationality of man. We must recognize that the greatest single danger to man's integrity and perhaps to our whole civilization is the subjugation of judgment by mass-media, before which even the perennial 'crooked questions' seem to be simplified for commercial or missionary ends; I mean those of love, death, pain, decay; of the essential dichotomy in the marriage relationship; of the existence of evil and tragedy; of the whole paradox of man's nature, the conflict between his 'erected wit' and his 'infected will', or St. Paul's great paradox (*Rom.* 7, 10). It is, I think, probable that we are to-day experiencing an induration or atrophy of the basic spiritual faculties; among those of sympathy, responsibility, and real (as opposed to fictitious or factitious) pleasure. And to this problem of sensitivity we must return.

VI

Now at this point I must attempt a brief excursion into metaphysics. Let us represent human experience by means of a tentative diagram, thus:



showing two poles or dimensions. The 'x' axis may be thought of as empirical in its nature; that is, it deals with the relationships and ideas that make up our normal common-sense world. Let *P* stand for the phenomena that compose our physical world. We may think of these as interrelated by laws of causality, themselves the proper objects of scientific inquiry. Let *E* be the ego which seeks to know the phenomena, which investigates and masters them. This horizontal axis of thought is Cartesian in origin. Along it moves pure reason which, rightly used, can solve all the riddles of the physical universe. Along this line the solution of the problem of the interrelations of all phenomena is possible. This solution is perceived as being in itself a 'good'.

Across this axis cuts the other[2]. Along it moves the 'community mind', *C*: which is not the 'mass mind' of war hysteria, or of popular riots. It is a mind which includes Jung's "collective unconscious", but is not limited to that concept, or to the analogous one of "The Great Memory" (as used, say, by W. B. Yeats). It grows slowly through the ages; it carries with it a store of collective wisdom in its myths. It moves on the vertical axis towards *M*, the sense of mystery in the world: and by mystery I mean those elements in the human situation which lend themselves only to intermittent apprehension, to which the approach cannot be made through rational or logical mechanisms; but which are of infinite importance in the total commitment of men.

I have very much in mind Jung's *Modern Man in Search of a Soul*; as well as the work of Joseph Wheelwright, Maud Bodkin, Austin Farrer and others. I believe passionately that the completed man needs the psychic satisfaction that is given by poetry, mythology, æsthetic experience: which, in many forms, can provide us with what Synge called "the nourishment, not very easy to define, on which our souls live". That this life of the imagination demands continually such nourishment, that it grows atrophied or indurated without it, I am certain. So long as there was a common ground of religious mythology (I prefer to use—following Jung and Kerényi—the word 'mythologem')—whether Biblical or Homeric—there was at least a common core of values, implicit or explicit, to be perceived in relation to them. The break-up of the common area of experience may perhaps be dated from about a hundred years ago, though something of it remained, I think, until 1914. We are familiar with *The Waste Land* of the 'twenties, the 'Pylon poets' of the 'thirties, the recurrent chorus that announced disintegration; and while the very act of perceiving that there is a disintegration is not without its value, we must clearly look for something more positive in character.

Now what this positive may be will depend, in the final analysis, on our own reading of the human situation. It seems to me improbable that poetry has much to give us if we accept wholly any of the empiricist philosophies, among them behaviourism that equates mind with the physical constitution of the body; instrumenticism which "reduces the meaning of any concept to that set of experimental operations by which the denotation of the concept can be objectively shown", and the fashionable semantic positivism which aims at a one-to-one correspondence between units of language and the sets or types of objects which such language units denote. Existentialism alone can point to a bitter ironical poetry of its own; since, while it jettisons many of the primary and traditional values, it holds fast to the status of the individual in his right of choice, in his need for integrity, nobility and courage; even though, to the existentialist, "life begins on the other side of despair".

Poetry does and must deal with the penumbral; and its penumbra may be the twilight world which reason and instinct, memory and present action, the conscious and the unconscious, merge imperceptively into each other. We read poetry because the inmost character of this penumbral world is only perceptible in the poetic statement, in the poem, in its own self-subsisting images, and not in any exegesis of it; because the fusion which it involves is its own highly complex justification; because its relationship to the totality of life is not perceptible without a study of its methods and techniques which can so easily atrophy, and which so often, in our present-day civilization, appears to give rise to a widespread aridity of consciousness.

It is at this point that I return to Shelley's

> "Poetry administers to the *effect* by acting upon the *cause*."

—that is, one operation of poetry is to produce, by exercising the creative imagination, the sense of awareness or sensibility which provides us with an extension of the individual towards unity with others and with the created world. Shelley in his time was arguing against the all-pervading rationalism, the *laissez-faire* economics of his time; and against that reason, unmitigated by pity, which had made "the rich become richer, and the poor become poorer". As he saw it, evil would in theory become impossible if all men were wholly sensitized and sensitive to the suffering of others; you do not do evil to others if your own imagination tortures you at the thought of their suffering. Perhaps this is what St. Paul meant by "charity", and some of us believe that various complex causes—among them the effects of two wars, the rise of the Welfare State, the rapid and violent re-orientation of communal values—have resulted in a diminished sense of pity and love, and perhaps of other virtues that are allied to these.

We can extend this thought a little towards the special problems of the scientist, whose work, habits of thought, pre-occupations with the *fact*, can and does distort or submerge, on occasion, his capacity for purely human relationships, whether in the family, in business, in the State or in international settings. I am clear that all the great scientists that I myself have known have sought and invariably achieved some balance, some counterpoise, through a form of art, practised or enjoyed, which has taken a prominent place in their lives: which seems to be the "nourishment, not very easy to define, on which our souls live". But one of them has suggested to

me a further consideration: the possibility that some of the 'great' discoveries may now be going unmade precisely because so many students of science to-day allow their imaginative powers to atrophy. I have used the terms 'nourishment', 'feeding', 'exercise'; for I believe in its need for these things the imagination does not differ in kind from any other mental power. But unless it is exercised it does die quickly away; and with it that mysterious power of making the creative 'leaps in the dark', the habits of synthesizing, finding relationships between disparate events, ideas, and above all, perceiving values through comparison. How far this may be true, none of us, are, I think, competent to say. I can only judge from case-histories within my knowledge; in which the tremendous and ever-increasing pressures on the natural sciences student seem often to make it impossible for him to expand his interests in a manner which would ultimately permit him to bring a trained and flexible mind to the forming of value-judgments, and which, perhaps, diminish the probability that his creative imagination will contribute to his discoveries.

I do not, myself, wish to suggest that there is any difference in kind in the potential or the sensibility of, let us say, the mathematician as contrasted with the arts man in forming these value-judgments. What divergence there is seems to me to arise because of the manner in which the arts student is brought into continual contact with historical, political, philosophical and religious problems (however tangential or partial they may appear) that are refracted through the material which he studies. He thus becomes aware that such problems are highly complex, involving a number of indeterminate factors, and admitting of no quick or easy answer. Some experience of European diplomacy in time of war seemed to suggest the dangers of seeking for solutions that should in theory have been clear-cut, rapid, rational; when they were in fact the products of a slow maturation in time, and of immensely complex human and national factors that were themselves rooted in history. The search for the quick, the black-and-white answer seems to be an eternal temptation of the immature, the man or nation that is in too much of a hurry: and this (though I do not propose to enter into that discussion) is not without relevance to the problem of policy-making, and of taking central or seminal decisions, in industry and in international affairs.

VII

At this stage it will be seen that I am making no special claim for poetry as opposed to any other form of art that serves to feed this creative imagination; remarking only on poetry's greater complexity and 'impurity' of statement as compared with the statement of, say, music or sculpture. I myself believe that poetry has suffered from the zeal, and sometimes the arrogance, of its fanatical supporters. I had some early training as a mathematician, and I went to Cambridge as a modern linguist. I yield to no one in my love of poetry, and I carried much of it in my head during the Second World War, in many countries and in many crises both of personality, diplomacy and war. But I do not believe in the possibility that it should ever become a significant formative force over any considerable area of the national consciousness. It has always been, and I think it will remain, of limited though immensely important appeal and incidence. For poetry demands, like any other art, effort and study: whether it be Shakespeare, or Donne, or 'difficult' modern poetry. In theory all art should offer a delight that is easy, quick and having some considerable degree of universality. No art since the sixteenth century (perhaps the fourteenth) has fulfilled those conditions.

VIII

My own brief summary would, I think, run something like this:

Poetry can communicate to us, in a very special manner, the collective wisdom of generations of men and women. These experiences are (this is the price of their survival) archetypal and perennial; since they are rooted deeply in the human situation, in the conscious and unconscious, in the past and present.

By the peculiar (and still largely unexplored) methods of communication which embody a combination of the semantic and phonetic aspects of language, and by its devices of rhythm, which are strictly related to the total statement made, it can achieve an acceptance of vicarious or stated experience in such a way as to secure its integration into the human personality. It may and does become one 'experienced' aspect of the 'armour against fate'; and this over an unimaginably wide range.

By its presentation of integrated, shaped and organized experience (a play is a convenient example of this rhythmic structure) it satisfies our need for the intermittent perception of a complete experience in a world which, without art, we feel to be fundamentally chaotic, inchoate.

By its feeding, exercising of the imagination—which I would define as that faculty which enables us to be aware of the immaterial in its infinite forms—poetry can fill that perennial human need for a life in which the mythic element can obtain the psychic satisfaction which it requires. I do not hold, with Matthew Arnold, that "the greater part of our religion to-day is its unconscious poetry"; for I do not believe that poetry can ever be a substitute for philosophy or religion; even to those who may believe, as good agnostics, that they have swept and garnished the last room of their minds, and are merely waiting . . . for what? I believe that poetry may prepare us for our journey into religion or philosophy, by just this process of sensitization, of increasing awareness, of which I spoke; I do not believe that it is a substitute. Nor do I believe (in contradiction to some of my colleagues) that a complete and implicit body of morality can or does arise out of poetry, since I believe that a body of moral belief, which must be won (at whatever cost) by the individual, is a prior necessity. I would argue that many of our problems to-day arise because of our attempts, in popular religions or in popular science, to short-circuit the long and painful processes of gaining knowledge and of adjusting that knowledge to the human situation. As for myself, I am certain that, if we are to allow the impact of any great art on us, we must be prepared to make certain assumptions (I am aware that many would give only a pragmatic assent, "the willing suspension of disbelief that constitutes poetic faith"), which I would set out as follows: (1) that there is a mind, or spirit, or soul, or psyche, which is not susceptible of explanation, as regards its powers and operations, on any known physio-chemical-biological hypothesis; (2) that the human situation involves the apprehension, intuitive in character, of a vast body of mystery,

which can be apprehended only by an intermittent and uncertain process of illumination, which grows, I think, less intermittent as we ourselves become older; (3) that poetry gives us one method of progress towards these apprehensions, and in particular of those that concern the penumbral—but not unfamiliar—worlds of which I have spoken; and which we exclude from our lives at our peril.

A quotation from Browning will serve:

"Just when we are safest, there's a sunset-touch,
A fancy from a flower-bell, someone's death,
A chorus-ending from Euripides,—
And that's enough for fifty hopes and fears
As old and new at once as Nature's self,
To rap and knock and enter in our soul,
Take hands and dance there, a fantastic ring,
Round the ancient idol, on his base again,—
The grand Perhaps! we look on helplessly—
There the old misgivings, crooked questions are— . . ."

IX

No more than this, you say? There is indeed; but my final judgment must be empirical, the result of watching many generations in their progress through literature and life. I am certain that the average undergraduate will one day be a better parent, lover, statesman, leader, for having read Shakespeare, Donne, Blake, Keats, Meredith. When he comes to his death he will die the better for having read, say, Dunbar, Donne, Herbert, Wordsworth. He will know better the essence of 'country'—and we neglect those roots at our peril—for having read Traherne, or Wordsworth, or Hopkins, or for having understood Constable's landscapes.

That is not to say that it would be better never to have been in love than never to have read Shakespeare. No literature of war can convey its full horror and exaltation and sense of futility until one has wasted years of life on it, or killed a man. Poetry, like all art, must demand and secure the perpetual opportunity to balance itself against physical life, experience; to test mutually the values thus discovered and embodied. Much of its disrepute is because of its claim to become an end in itself, the plaything of æstheticism, the spell-bound Lady of Shalott, the Sleeping Beauty in the jewelled sensual cave, or far worse than these, the esoteric speech of "the professors whose mouths are gorged with sawdust". Perhaps all that I am trying to say is summed up in an adaptation of a text that you will recognize. Its function is to give life, the strange and vitally energizing life of the psyche; and to give it more abundantly.

[1] Richards, I. A., *Science and Poetry*.
[2] I am indebted to *The Language of Poetry*, edit. by Tate (J. Wheelwright's article).

A STRATEGY FOR THE WAR ON HUNGER

By Sir JOSEPH HUTCHINSON, C.M.G., F.R.S.
University of Cambridge
and
Prof. A. H. BUNTING
University of Reading

THE Rockefeller Foundation instituted a public health programme in Mexico in 1919. By 1943, rising health standards there had produced one of the great modern human problems—the problem of feeding the rising population produced by scientific death control. In that year the Foundation and the Mexican Government inaugurated a co-operative programme in agricultural research which has served as a model for similar operations in other countries. Aided by this programme, an average annual population increase, during the past ten years, of about 3 per cent has been over-matched by an average annual increase of 7 per cent in food production. Seven per cent is one of the highest rates of increase of agricultural production recorded anywhere in the world in this period, but it is perhaps relevant to point out that while from 1948–52 to 1957 rice production in Asia increased at about 3·7 per cent per annum, in mainland China the figure was 5·7 per cent (calculated from *F.A.O. Mon. Bull. Statistics*, Dec. 1959).

Co-operation between the Foundation and the Mexican Government was based on a jointly supported and staffed operating unit, the Office of Special Studies, which was set up in 1943 within the Mexican Ministry of Agriculture. The Office of Special Studies has operated a number of research stations and has directed the scientific work in others. In the first instance the Foundation provided senior staff. From the beginning, they undertook the training of Mexican graduates, first on the stations in Mexico, and later in American universities. In this way fully trained Mexican staff became available to replace Americans in senior posts. In fact the development of the work has led to a continuing demand for senior American staff; but the proportion of the senior staff who are Mexican has steadily increased. In 1959–60, of a staff of 147, more than 130 were qualified Mexicans, and in 1960 it was possible to plan the unification of the Office of Special Studies with the Mexican national organization for agricultural research.

This general pattern of activity was repeated in Colombia from 1950, in Chile from 1955 and in India from 1956. Two international programmes have evolved from these experiences. The Inter-American Food Crop Improvement Program has been established to apply the collective experience of the Americas to food-crop research and production (specializing initially on wheat and maize), particularly in those areas of Latin America which do not have operating units of the Foundation. The International Rice Institute in the Philippines, which has also been aided by the Ford Foundation, is a joint enterprise of several rice-growing countries of south-east Asia.

XUM

The Rockefeller Foundation has now issued the first report of these activities*. Though nominally confined to a single year, it gives a clear account of the development of the programme and of its methods of work.

The programme is primarily agronomic in the broad American sense, covering the selection and breeding of food crops, forage plants, vegetables and stock, work on plant and animal pests and disease, on soils, and on weed control. If we add to this list agricultural economics and extension methods, we can discern the usual, and very effective, pattern of agricultural activity at State-level in the United States, where research is closely linked with the educational system and with the Federal agencies for more general or more fundamental studies. The programme clearly benefits greatly from the experience of the United States in handling the problems of areas of great geographical diversity, and from the great plant collections assembled by the United States Department of Agriculture. For example, the Inter-American Food Crop Improvement Program uses standard sets of wheat varieties, prepared by the U.S. Department of Agriculture, in different areas to determine the best-adapted types for different localities, in relation both to yield and rust resistance. A standard set of United States maize hybrids has been of great use, in the early stages of maize improvement work in various countries, in indicating the general varietal types most likely to succeed.

It is impossible here to summarize in detail the mass of useful work reported from so many regions; but a few of the points which seemed especially noteworthy may be quoted.

The largest single activity is the improvement of maize, followed closely by that of wheat and sorghum —three crops of which the United States has unique experience. Hybrid maize naturally occupies a central place. A hybrid maize programme has made remarkable progress in India, where double hybrids were to be released in 1960 for most of the maize-growing regions. In Mexico, a series of crosses between Mexican tropical varieties of maize and other Central American and U.S. material has initiated the modern exploitation of the vast genetic reservoir in the Latin American races of this crop. Work on the isolation of superior material by the testing of S_1 lines holds promise for the speedy improvement of indigenous maize. Remarkable progress, based on the American genetic collections, has been made in the improvement of sorghum and wheat. From the same country it is reported that "minute quantities" of potassium gibberellate (a spray of 1 p.p.m. at an unspecified rate) increased the seed yield of beans (*Phaseolus vulgaris*), while high-yielding bean hybrids will probably be released in 1962. A naked-seeded squash for oil extraction has been isolated. A blight-resistance breeding programme in potatoes has been based on *Solanum demissum*.

In Colombia, at Medellin, nearly 6,000 indigenous South American strains of maize, mostly from the Andes, are being preserved, partly in race composites (panmixis bulks), for breeding and research. Accounts of the races of maize in Chile, Venezuela and Ecuador will shortly be published, so that the indigenous maize of the greater part of South and Central America will then have been recorded by various hands. A new source of male sterility has been found in Colombian maize, and the development of broad-based synthetics for areas of less-advanced agriculture is foreshadowed. Important work on potato breeding using species other than *S. tuberosum* is also reported.

The most striking feature of the programme in India, where the Foundation has only six resident staff members, is the inauguration, in October 1958, of the postgraduate school at the Indian Agricultural Research Institute. In 1959–60 there were 186 M.Sc. and 113 Ph.D. students, following typically North American courses combining class-work and research training. The future intake is to be at the rate of 100 M.Sc. and 50 Ph.D. students a year. To write off this great educational effort as merely factory production of technologists would be to miss one of the most important features of such an enterprise in a country where there are many graduates but few trained and experienced research workers. The gap between a first degree and a fully trained research worker is extremely difficult to bridge in such circumstances, and the difficulties of the situation are enhanced by the problem of varying standards in the local universities. Such a postgraduate school is required not only to provide the advanced training that is necessary but also to give that degree of individual attention that is necessary if men of very different initial standards are to be turned out with a fair degree of comparability in experience and attainment. There is no doubt that the Indian Agricultural Research Institute postgraduate school will go far to meet a very real need.

The achievements of the Rockefeller programme must pose many questions for British readers. On a much smaller scale, the Empire Cotton Growing Corporation (also an unofficial organization co-operating with Governments) is in many ways comparable. Based on the genetic improvement of the cotton crop, its activities cover a wide agronomic range in several tropical territories, and in at least one it provides the backbone of agricultural research. Yet in territory after territory with which Britain has historical and still friendly links, it is proving extremely difficult to meet the demand for agricultural research workers and teachers. The Sudan Agricultural Research Division, once the finest organization of its type in tropical Africa, is at present seeking senior staff. For some years past western Nigeria has been attempting to recruit senior staff to build up agricultural research. At least two chairs of agriculture are vacant in English-medium tropical faculties, others have not been easy to fill, and several more are shortly to be created. How is it, that after sixty years of effective and successful agricultural research in all regions of the world, and a leading position in the related basic sciences, it is so hard to find British nationals for this work? Why, in India of all countries, was it necessary for an American organization to develop a postgraduate school in agronomy?

There are perhaps three groups of reasons. One is historic: the ending of the imperial regime usually means a feeling of insecurity for British employees, while the demand for improved educational facilities for the children of expatriates and for greater amenities for their wives often presents real difficulties.

The second and far more serious reason is that we are not producing enough of the right people. In England and Wales we have now virtually completed the deliberate and formal separation from each other of agricultural advice, education and research, the unity of which provides one of the great strengths

* The Rockefeller Foundation, 1961. *Program in the Agricultural Sciences.* Annual Report 1959–1960. Pp. ix + 292 + many plates and diagrams. (New York: Office of Publications, The Rockefeller Foundation, 1961.) n.p.

of the American system. In no English university is there a field of real-life activity within which an agronomist in the broad sense can be trained. None of the university faculties of agriculture is large enough to provide the necessary range, especially since the advisory service, with its husbandry farms, has been separated from the universities. In some university departments, the trend of research interest, which inevitably comes to dominate teaching, is consequently forced away from the primary analysis and solution of practical problems towards narrow specialization and academic fundamentalism. In many agricultural research institutes it is very difficult for young research workers to qualify for higher degrees. Whatever one may think of the relative values of published papers and paper qualifications, it is undeniable that the discipline and criticism of a well-conducted higher degree course can play an important part in a research apprenticeship. Few British universities and research institutes have an active interest in tropical countries; neither the Agricultural Research Council nor any British university has a tropical field station for research on crops and soils; and no reciprocal arrangements for teaching or research exist between tropical centres and their British counterparts.

Thirdly, there are administrative reasons of which one or two may be mentioned. Secondment arrangements for tropical service and experience are rare, though secondment would often be possible without cost to home institutions, and secondment contracts of a suitable type could be a powerful aid to recruitment for overseas service. The formation of the Overseas Research Service does not yet appear to have increased the stability or sense of career security of overseas staffs, nor is the Overseas Research Council playing any effective part. The new Technical Assistance Department has an inspiring task and good leadership; if it can steer a way through the jagged reefs of its terms of reference it could have a great future—provided its course is outwards, to the deep blue waters of the stormy ocean, and not inwards to the sheltered calm of the lagoon.

This report of the Rockefeller Foundation under review can be recommended as wholesome and salutary reading for all who are concerned with the British effort in developing countries. United States participation in this field is increasing; and so powerful a reinforcement must be sincerely welcomed, particularly if it leads to a clear-sighted re-appraisal of the British position, and provides a stimulus to Britain's future efforts.

OBITUARIES

Sir K. S. Krishnan, F.R.S.

THE sudden death on June 14 of Sir K. S. Krishnan, director of the National Physical Laboratory of India, at the age of sixty-two, is a grievous loss to Indian science in which he has been a leading figure for many years. Following his training at the American College, Madura, the Christian College, Madras, and the University College of Science, Calcutta, and a short period as demonstrator in chemistry at Madras, he joined the Indian Association for the Cultivation of Science, Calcutta, as a research associate in 1923. In association with C. V. Raman (later Sir C. V. Raman) he undertook a variety of researches in optics culminating in the discovery of the Raman effect in 1928, which was not only of immediate significance in itself, but also proved later to be an important tool for the study of molecular structure.

Shortly after, he became reader in physics in the University of Dacca and began a systematic study of the magnetic properties of dia- and para-magnetic crystals. This work continued and indeed bore its main fruit when he returned to the Indian Association as Mahendralal Sircar research professor in 1933. It is described in a series of papers, published in the *Philosophical Transactions of the Royal Society*, by Krishnan and his students during the 1930's. These are distinguished by the elegant simplicity of the experimental methods described (mostly devised by himself), and by the ingenuity of interpretation and the clarity of exposition. The results threw much new light on questions lying on the border between chemistry and physics, such as the arrangement of molecules in organic crystals, the structure of molecules, and the influence of crystalline fields on the paramagnetism of ions. During 1942–47 he was professor of physics in the University of Allahabad, and while still continuing experimental work on optics and magnetism began to interest himself more in theoretical problems such as the resistivity of solid and liquid metals. His election as Fellow of the Royal Society in 1940 and his knighthood in 1946 were very well-deserved recognitions of his scientific achievements.

In 1947 he was appointed director of the newly created National Physical Laboratory of India and with vigorous support from the late Sir S. S. Bhatnagar nursed it through its teething troubles to make it one of the largest and best-equipped scientific institutions of India. The award of the first Bhatnagar Medal to Krishnan shortly before his death was particularly appropriate and gave him much pleasure. During this time Krishnan gradually became one of the elder statesmen of Indian science: no official committee was complete without him, and he was much in demand for opening ceremonies and took an increasing part in international scientific affairs. He was, for example, vice-president of the International Council of Scientific Unions from 1955 and of the International Union of Pure and Applied Physics during 1951–57, and chairman of the Unesco Scientific Advisory Committee in 1955.

However, it must be admitted that though he felt keenly his responsibility as a public figure, administration was not really to his taste. In spite of all the calls on his time, he managed to keep going some active theoretical research of his own, to cultivate his mathematical talents and to read widely in many fields, by no means confined to science. I had the good fortune to spend a year at the National Physical Laboratory a few years ago, and the informal chats I had with Krishnan, particularly over lunch in the canteen, provide some of my most vivid recollections of that time. He never seemed to forget anything he had once read or heard, and had an encyclopædic

knowledge in all sorts of unexpected fields. To illustrate an argument he was fond of quoting anecdotes about the great classical figures of physics, and this he could do in the most entertaining way. But he was equally at home in the latest developments of science and was a shrewd commentator on people and politics.

All this erudition was combined with great charm, good humour and kindness. His kindness, however, had its drawbacks when it came to administration, for he could not bring himself to say anything which would disappoint his hearer, and preferred to defer a difficult decision indefinitely rather than cause anyone disappointment. Another idiosyncrasy was that he could scarcely ever be persuaded to write a letter; thus a visit from him usually came as a surprise, preceded at the best by a telegram, but more often introduced by an apologetic chuckle as he explained that he had meant to write. His genial presence both at home and abroad will be sadly missed by a host of friends. He leaves a widow, two sons, four daughters and three grandchildren.

D. Shoenberg

Dr. N. S. Shatsky

Dr. Nikolai Sergeevich Shatsky, member of the Academy of Sciences of the U.S.S.R. and director of the Institute of Geology, died in Moscow in November 1960. He was born there on August 28, 1895. He began his geological career as a stratigrapher, carrying out a detailed geological survey in the Donetz basin, and in the Caucasus and Volga regions. This work led him to the study of geological formations as stratigraphical units. At the same time, Shatsky was writing of oil and coal-bearing strata, iron ores, manganese ores and phosphorites. It was, however, in the field of tectonics that Shatsky excelled, and it is not without reason that he is considered to be the founder of a new tectonic school in the U.S.S.R.

His tectonic and palæogeological investigations in the European and Asiatic parts of the U.S.S.R. led him to establish a new Upper Proterozoic system, which he named the Rhiphæan system, and a new orogenetic epoch, coeval with this system, which he named the "Baikalian" epoch. However, his greatest achievement, shared with numerous collaborators, was a tectonic survey of the whole territory of the U.S.S.R., leading to the publication in 1956 of a most original, and in a sense most beautiful, tectonic map of the U.S.S.R. on the scale of 1 : 5,000,000. This map was the first of its kind. In it different colours were used for the major orogenic epochs and a number of tectonic structures were indicated by special signs. The Rhiphæan system and the tectonic map were briefly reviewed in Britain (*Proc. Geol. Soc., Lond.*, No. 1501, 108; 1953; and No. 1557, 29; 1958). Shatsky was also interested in the history of geology, and in 1941 he published, in Russian, a book on the life and work of the famous British geologist, R. I. Murchison, who greatly advanced the progress of geology in Russia. In March 1960, Shatsky was elected an Honorary Fellow of the Geological Society of London.

S. I. Tomkeieff

Mr. B. Welbourn

Mr. B. Welbourn, formerly director and chief engineer of British Insulated Cables, died on July 1 at the age of eighty-five. He retired in 1945 on the formation of British Insulated Callender's Cables, Ltd., through the amalgamation of the British Insulated Co. and Callender's Cable and Construction Co.

Mr. Welbourn graduated from King's College, London, in 1896. After a period of service with the Electrochemical Co. of St. Helens, he joined, in 1897, the British Insulated Wire Co., of Prescot, becoming contracts manager in 1902, in which capacity he travelled widely. In 1927 came his appointment as chief engineer, and in 1942 he was elected to the board of the British Insulated Co.

In a life-time spent in the electrical industry, in which he had such a notable career, Mr. Welbourn had served as a member of the Council of the Institution of Electrical Engineers, was a vice-president of the Institution and past chairman of the Manchester and Liverpool centres.

NEWS and VIEWS

Canadian Scientific Attaché in Washington: Dr. E. O. Hughes

Dr. E. O. Hughes, of the National Research Council of Canada, has been appointed as scientific attaché to the Canadian Embassy in Washington. While in Washington, Dr. Hughes will also serve as Canadian liaison officer at the British Commonwealth Scientific Office. Born in Wales, in 1916, Dr. Hughes received his initial education in Winnipeg, Manitoba, and Halifax, Nova Scotia. After undergraduate training in biology at McGill and Dalhousie Universities, he received an M.A. degree in 1939 from the University of Western Ontario. In 1942 he was granted a Ph.D. degree at the Ohio State University. During 1942–45 he was engaged in the administration of medical research for the National Research Council of Canada and the Royal Canadian Army Medical Corps. In 1945, Dr. Hughes became assistant professor of plant sciences in the University of Oklahoma, Norman, Oklahoma. In 1952 he returned to Canada as a research biologist and editor in the Division of Applied Biology of the National Research Council. His work was primarily concerned with the classification and physiology of freshwater algae. During 1956–60 he was assistant to the director of the Biology and Health Physics Division of Atomic Energy of Canada Limited, Chalk River, Ontario. For the past year he has been acting chief of the public relations office at the National Research Council of Canada.

C.S.I.R.O. National Standards Laboratory : Mr. N. A. Esserman

Mr. Norman A. Esserman, director of the National Standards Laboratory of the Commonwealth Scientific and Industrial Research Organization (Australia), has retired after twenty-three years service. Mr. Esserman graduated from the University of Sydney in 1916 with first-class honours in mathematics. He went to England in 1917 and worked in the Arsenal Branch of the

Munitions Office. On returning to Australia in 1920 he joined the Department of Defence, and afterwards became assistant superintendent of the Munitions Supply Laboratories (now the Defence Standards Laboratories) at Maribyrnong, Victoria. In 1938 he joined the Organization as officer-in-charge of the Metrology Section, one of the three units of the National Standards Laboratory. It was largely due to his experienced guidance that the Section was able to establish itself quickly and to make an outstanding contribution to Australia's war-time production. In 1958 he was appointed first director of the National Standards Laboratory. Mr. Esserman has been personally honoured by his election to the International Committee for Weights and Measures—a fitting climax to a career devoted to physical measurements and a tribute to the man who has had the unique distinction of playing the leading part in establishing the two main laboratories in Australia concerned with metrology.

C.S.I.R.O. Division of Mineral Chemistry : Mr. I. E. Newnham

Mr. Ivan E. Newnham has been appointed chief of the Division of Mineral Chemistry, Commonwealth Scientific and Industrial Research Organization (Australia). He is forty-one years old and graduated M.Sc. from the University of Melbourne in 1940. In 1942 he joined the Department of Aircraft Production and afterwards became supervisor of the Department's Testing Laboratories, where he was associated with the production of the first aircraft bearings made in Australia. During the later war years, while employed by the Newcastle firm of P. J. Taylor Pty., Ltd., he pioneered new techniques for the manufacture of automotive bearings. Since joining the Organization in 1947, Mr. Newnham's main interests have centred around the chemistry of the metals zirconium, hafnium and beryllium, all of which occur in Australian minerals. During 1950–55 he was engaged on research into methods for separating zirconium and hafnium, which occur together in the mineral zircon, in Australian beach sands. Pure zirconium is used as a container for uranium in the manufacture of atomic reactors, especially for use in nuclear submarines. Hafnium, which absorbs neutrons, is used for making control rods in reactors.

Physics at Sheffield: Prof. N. H. March

Dr. N. H. March has been appointed to the newly created second chair of physics in the University of Sheffield. He graduated at King's College, London, in 1948, and there began his research career under the direction of Prof. C. A. Coulson, then professor of theoretical physics at King's College. In 1950, March was appointed to an assistant lectureship in physics at the University of Sheffield, promoted successively to the grade of lecturer in 1953, and reader in theoretical physics only four years later. He has been an enthusiastic research worker from the beginning, and soon came to be regarded as one of the recognized authorities on the Thomas–Fermi statistical theory. In addition, he has maintained a lively interest in the electronic structure of molecules, in particular the electrical charge distribution. More recently, probably not uninfluenced by the activities of his experimental co-workers in Sheffield, he has turned to problems connected with the solid state. Here he has made important contributions to the distribution of charge around impurity centres. He now leads an active group of postgraduate research workers, and his school can confidently be expected to make substantial growth with the increased accommodation shortly to be available for the physics department in Sheffield.

Promotion of Science in Australian Schools

The fund which was established in England in November 1955, by a group of industrial organizations, to assist in increasing the number of scientists and technologists at the service of industry and of the country generally, has led to the establishment of a similar fund for a similar purpose in Australia. It was inaugurated at a meeting of industrial and commercial leaders, held in Sydney on March 20, 1958, under the chairmanship of Sir Edward Knox, and publicly announced on February 2, 1960. The Fund seeks to pursue its objects by stimulating and encouraging the teaching of science in suitable schools. Prior consideration is being given to independent boys' schools of standing, and the schools which are represented in the Headmasters' Conference of Australia are recognized as coming within this definition. It is intended to extend the range of consideration if sufficient funds become available. The Fund is now proceeding to assist chosen schools in the building of up-to-date laboratories and other rooms for the teaching of science. Attention is being given to the basic sciences, namely, physics and chemistry, and geology in suitable cases. Assistance to other sciences is not at present contemplated. In their discussion with schools, the Fund's representatives have endeavoured to encourage schools to plan with a long view so that it will be easy to add further accommodation later. The hope has been expressed that schools will endeavour to encourage a greater volume of science at the upper levels of secondary education and that they will seek to stimulate work of a more advanced standard. Contributions made or promised to the Fund to December 31, 1960, total £576,315. Although the amount is considerable, it is still far from sufficient to realize the Fund's full objectives, and more help is needed from others in the fields of industry and commerce. Further information about the Fund is described in a report which may be obtained from the Secretary, M.L.C. Building, Petrie Street, Canberra.

Shipbuilding Research in Britain

In the debate on shipping and shipbuilding in the House of Commons on July 13, the Minister of Transport, Mr. E. Marples, agreed that Parliament should attach the very greatest weight to research and development for both the shipping and shipbuilding industries. Better co-ordination is required in the future, he said, and shipbuilding research and shipping research should not be isolated departments but more and more together; this is now being discussed with the General Council of British Shipping. Referring to the loss of orders by the industry, to the charges of lack of modernization, inefficient management, demarcation and restrictive practices by the unions as well as to insufficient research and development, he said that a new and dynamic approach to production is required. The future of the industry is at stake, and for some time discussions have been proceeding between the two sides of industry about ways and means of improving its efficiency and removing obstacles to progress. If ths discussions do not achieve substantial progress in the near future, the Government intends to review the position with both sides of industry. Mr. Marples

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stressed the need for co-operation by both sides: without it we must fail. The question of research was particularly emphasized by Mr. A. Albu, and, in replying on the debate, the Parliamentary Secretary to the Ministry of Transport, Vice-Admiral J. H. Hallett, said that the British Shipbuilding Research Association is ably directed and undertakes research into production methods and techniques. It is hoped that the two industries will now jointly evolve some wider research organization which can handle problems of operation and of design and building as a whole, including possibly research on Diesel engines.

The Institution of Electrical Engineers

Much of the scientific work of the Institution of Electrical Engineers has been for many years conducted by four specialized sections (electronics and communications, measurement and control, supply and utilization). It has now been decided that it will be advantageous for the four sections to be replaced by three divisions, two of which will represent electronics and power interests, while the third, a general division, will cover activities of common interest to all electrical engineers, such as basic measurement, and technological education. Each of the divisions will comprise a number of technical groups designed to cover the specializations within its field, and the scheme provides for speedy re-adjustment and creation of those technical groups which will increase the flexibility which is now more than ever necessary to reflect the rapid developments of electrical science and engineering. In setting up a divisional structure, the Council has also recognized the increasing importance of electronics in electrical engineering, and the part being played by electronics engineers in the work of the Institution which has resulted in its present position as the professional home of electronics engineers.

Australian Journal of Experimental Agriculture and Animal Husbandry

This new journal is devoted to reports both on agronomy and animal husbandry. Judging from the contents of the first number, the journal is likely to cover a wide range of topics: it includes articles on the potassium content of some Western Australian soils, legumes in pastures, classification of Australian wheat varieties based on the granularity of their wholemeal and the relationships between digestibility of dry matter and digestibility energy content of ruminant diets. A pleasing feature is the inclusion of summaries of each paper in the list of contents—a departure which might be more widely followed. The journal is published quarterly by the Australian Institute of Agricultural Science for the Australian Agricultural Council and the subscription is £A.5 a year including postage.

Index Biologicus

Under the title *Index Biologicus* a periodical is being planned that will be a classified index of current papers from international literature in pure biology, giving titles, authors and journal references. It is intended that immediate notice will be given of all papers published in this already very extensive and rapidly expanding field, and that biologists will be provided with an information service comparable with those now available in other subjects, for example, *Index Medicus*, *Current Chemical Papers* and *Chemical Titles*. Scientific direction of the journal will be the responsibility of Biological and Medical Abstracts, Ltd., a non-profit company, limited by guarantee, that was formed by a number of learned societies when *British Abstracts* ceased publication so that the physiological and biochemical sections might be carried on. Like the present *International Abstracts of Biological Sciences*, which is the successor to Section *A* III of *British Abstracts*, the *Index Biologicus* will be published on a contractual basis for Biological and Medical Abstracts by the Pergamon Press. It is hoped that this publication will meet a real need and, moreover, it is expected that co-operation in the framing of scientific policy will be forthcoming from biological societies in Britain and overseas. The first issue of the journal is planned to appear in June 1962 or January 1963. Any comments or inquiries should be made to Dr. R. K. Callow, chairman, Biological and Medical Abstracts, Ltd., 4 Fitzroy Square, London, W.1.

Museum of English and Rural Life

The annual report for 1960 of the Museum of English Rural Life at the University of Reading welcomes an acquisition from the British Council of some 300 examples of traditional design and fine workmanship (Pp. 19. 5 plates. Reading: The University. 1961. 1*s*.). The collection includes saddlery, wrought ironwork, small stools, turnery, basket work, pottery and textiles, and is of special value because the names and locations of the makers were known. More storage space has helped the re-arrangement of the reserve material, and increased workshop facilities have speeded the restoration of the larger farm implements and vehicles. The report of this organization is always noteworthy for the inclusion of notes on policy and other matters affecting the museum movement. On this occasion the curator, Mr. C. A. Jewell, contributes notes for collectors concerning the acquisition of folk-life material. It is rightly stated that traditional rural life in England was based on a relatively close-knit and self-sufficient community often with a distinct regional character. Although this has largely disappeared, scattered evidence still survives and practical and useful details are given on the collecting of objects and recording of information. The report is well illustrated.

Society for Folk-life Study

Plans to form a Society for Folk-life Study will be discussed at a conference to be held in London during September 6–8, 1961. Membership will be open to all who are interested in the traditional patterns of life in both rural and urban communities, and especially in local dialects, crafts, folk-lore and customs. It is intended that the new Society will serve as a focus for the activities of all who take an interest in their cultural inheritance. Various well-known experts in this field of study have promised to speak at the conference. Further information can be obtained from Mr. J. G. Jenkins, St. Fagans, nr. Cardiff, who is the secretary of the steering committee.

The Nitrogen Problem

Prof. N. R. Dhar, in his presidential address to the forty-eighth Indian Science Congress held at Roorkee this year, reviewed the importance of nitrogen in crop production. He pointed out that synthetic nitrogen plants have been started in many

industrially undeveloped countries, but they are handicapped by the heavy capital expenditure. The world demand for nitrogen fertilizers in 1960-61 is expected to be 8·1 million tons, of which Europe and the United States will together use 5·5 million tons. Prof. Dhar emphasized the desirability of building up phosphate reserves in the soil because natural fixation of atmospheric nitrogen in the soil is increased by adequate supplies of phosphate. Nitrogen is the key element in crop production; it is calculated that 100×10^6 tons of nitrogen are necessary for producing the world's food materials, excluding grass; but the chemical industry at present produces only 7×10^6 tons and legumes 5×10^6 tons. The nitrogen requirement of the world's grassland is estimated at 155×10^6 tons per annum, so that of the 250×10^6 tons of nitrogen necessary for the production of the major food crops of the world only 3 per cent comes from artificial fertilizers, 2 per cent from legumes, 2–3 per cent from precipitation and 2 per cent from organic manures. The rest is produced in the soil by microbiological activity on organic matter. Thus, industrial nitrogen supplies only a small part of the nitrogen requirement of crops, and a great deal can be done to increase land fertility by the better use of organic material supplemented by phosphate fertilizers to increase natural nitrogen fixation.

New Zealand Phytochemical Survey

DURING a survey of the New Zealand native flora for possible tumour inhibitory principles, extracts have been examined for the presence of alkaloids, leucoanthocyanins, saponins and triterpenes or steroids. In the latter work numerous fresh plants which are not extracted in bulk, and also herbarium specimens from the Auckland Institute and Museum, have been examined. The results of testing the New Zealand gymnosperms have now been reported (*N.Z. J. Sci.*, 4, No. 1; March 1961). The investigations show that alkaloids are not commonly found in species of the gymnosperms, notable exceptions being ephedrine from *Ephedra* species and taxine from *Taxus baccata*. No species of the New Zealand Cupressaceae or Podocarpaceae were found to contain alkaloids, but the heartwood of the sole representative of the Pinaceae, *Agathis australis*, was found to give positive tests. The presence of an alkaloid has been afterwards confirmed when bulk samples of the heartwood were extracted and a water-insoluble alkaloidal fraction isolated in low yield. Attempts to isolate a pure compound have been unsuccessful. All the species gave positive tests for leucoanthocyanins, the barks of the plants being the richest source of these compounds. The isolation of the principle leucoanthocyanin from the bark of *Dacrydium cupressinum* has already been reported and has been shown to be a stereoisomer of leucocyanidin which is apparently not identical with any isomer previously isolated. That from the bark of *Dacrydium biforme* has also been isolated by one of the investigators but has not been obtained pure. It differs from that of *D. cupressinum*, giving delphinidin on hydrolysis.

Data on Research and Development

A PRELIMINARY report issued by the National Science Foundation (*Reviews of Data on Research and Development*, No. 28; June 1961. *NSF* 61–31) gives the capital expenditure of colleges and universities in the United States for research and development in the natural and social sciences in 1958 as 153·5 million dollars, of which 112 million dollars came from non-Federal sources. The remainder came from the Federal Government, being largely confined to the large research centres which colleges and universities administer for the Federal Government. Half this expenditure was in the biological sciences, 33 per cent in the physical sciences, 15 per cent in the engineering, and 3 per cent in the social sciences.

J.L.T. Industrial Research Fellowships

J. LANGHAM THOMPSON, LTD., are introducing a scheme of fellowships for young research workers, normally on a two-year tenure, with the intention that those appointed should carry out their research in the Company's laboratories in Watford, Hertfordshire. The fields of interest to the Company include: solid state, cybernetics, logical systems, electronics, circuit techniques, micro-waves, mechanics of solids, transducer techniques, optics. Candidates for these fellowships should have completed a higher degree, or have carried out original research of a high standard and have a clear appreciation of the research programme they wish to pursue. Further information can be obtained from J. Langham Thompson, Ltd., 176 High Road, Bushey Heath, Hertfordshire.

Sixth International Mineral Processing Congress

THE sixth International Mineral Processing Congress will be held in Cannes during May 26–June 1, 1963. Organization is in the hands of la Société de l'Industrie Minérale and the following executive committee has been appointed: *President*, M. R. J. Testut; *General Secretary*, M. Pierre Gy; *Members*, MM. Astier, Emery, Ploix, Rey, Saint-Guilhem, Saverot, Seyer, Vielledent and Vuchot. As in the past, this Congress will be concerned with all aspects of mineral processing, and papers will be selected entirely on the basis of their scientific and technical value. However, the Committee wishes to place particular emphasis on the following subjects: (1) the concentration of iron minerals, including magnetic roasting but excluding agglomeration; (2) measurement, control and automation; (3) hydrometallurgy. Especial importance is also attached to the economic aspects of mineral processing and it is hoped that, whenever possible, authors will include data on installation and operating costs and comparative costs for alternative processes. The official languages of the Congress will be French, English, Russian and German, and provision will be made for simultaneous translation of discussions. An extensive series of visits to mineral processing plants and laboratories in Mediterranean countries will be arranged. The representative for the United Kingdom and the British Commonwealth is Prof. M. G. Fleming, Royal School of Mines, Prince Consort Road, London, S.W.7, to whom abstracts must be sent by October 15, 1961.

University News : Birmingham

PROF. WILLIAM HENRY TRETHOWAN, professor of psychiatry in the University of Sydney, has been appointed to the chair of psychiatry; the date will be arranged.

Edinburgh

THE following appointments have been announced: *Imperial Chemical Industries Research Fellows*, Juris Reinfelds (Adelaide) and Imre Laszlo (Debrecen),

Lectureships. R. F. Thow (agriculture); Dr. L. M. Muggleton (electrical engineering); Benjamin Mitchell (veterinary surgery).

Leeds

New courses, leading to honours and ordinary B.Sc. degrees in food science, have been introduced, and the first students for them will enter the University in October 1962. The honours course extends over four years, and includes chemistry, physics, biology, statistics, bacteriology and biochemistry, taken in the appropriate pure science department in the first year. This is followed by courses in the chemistry, physics and microbiology of foodstuffs and food processing. The ordinary degree course is restricted to three years. Entry requirements include a high standard in chemistry and physics, with mathematics as the preferred third subject. The Procter Department of Food and Leather Science, under the Procter professor, A. G. Ward, has grown from the former Department of Leather Industries and will receive extended premises and additional staff for the new course. Postgraduate students will be able to take the postgraduate diploma in food science, involving study and research, or carry out research for the M.Sc. and Ph.D. degrees.

London

Mr. S. B. Watkins, head of the Department of Chemical Engineering at King's College, has been appointed to the University readership in chemical engineering tenable at that College. The title of reader in pure mathematics has been conferred on Dr. K. W. Gruenberg in respect of his post at Queen Mary College.

The title of professor emeritus has been conferred on the following: Prof. R. O. Buchanan, professor of geography in the London School of Economics and Political Science since 1949; Prof. L. P. Garrod, professor of bacteriology in St. Bartholomew's Hospital Medical College since 1937; Sir Austin Bradford Hill, professor of medical statistics at the London School of Hygiene and Tropical Medicine since 1945, and honorary director of the Medical Research Council's Statistical Research Unit; Sir Christopher Ingold, professor of chemistry in University College since 1930; Prof. W. H. Linnell, professor of pharmaceutical chemistry in the School of Pharmacy since 1944, and dean of the School; Prof. C. A. Mace, professor of psychology in Birkbeck College since 1944; Prof. D. M. Newitt, Courtauld professor of chemical engineering in the Imperial College of Science and Technology since 1945; Prof. F. R. Winton, professor of pharmacology in University College since 1938.

Announcements

The Earl of Halsbury, during 1949–59 managing director of the National Research Development Corporation, has been elected president of the Machine Tool Industry Research Association.

Dr. F. A. Robinson, at present director of research of Allen and Hanburys, Ltd., has been appointed chairman of the board of directors of the Crookes Laboratories in succession to Mr. N. B. Smiley, of Arthur Guinness, Son and Co., which acquired the Crookes Laboratories jointly with Philips Electrical Industries last November. Dr. Robinson is honorary treasurer of the Biochemical Society and the author of books on chromatography, antibiotics and the vitamin B complex.

Colonel J. F. Williams-Wynne has been appointed a member of the National Parks Commission. Colonel Williams-Wynne is a part-time member of the Merseyside and North Wales Electricity Board and a member of the Regional Advisory Committee of the North Wales Conservancy of the Forestry Commission and the Gwynedd River Board.

Lord Shawcross has been appointed a member of the Medical Research Council. He will serve as chairman of the Council in succession to Viscount Amory, who is resigning on his appointment as United Kingdom High Commissioner in Canada.

Mr. A. W. Jones (Fleming Radio (Developments) Ltd.) has been installed as president of the Scientific Instrument Manufacturers' Association of Great Britain for the year 1961–62 by Mr. G. C. Ottway (W. Ottway and Co., Ltd.), the retiring president who now becomes a vice-president. The following honorary officers have been elected: *Vice-President and President-elect*, Mr. R. E. Burnett (Marconi Instruments, Ltd.); *Vice-President*, Mr. G. C. Ottway (W. Ottway and Co., Ltd.); *Secretary*, Major W. Logan (Avo, Ltd.); *Treasurer*, Mr. G. S. Sturrock (Kelvin and Hughes, Ltd.).

To celebrate the third centenary of the birth of Antonio Vallisneri, a past-president of the "Accademia Patavina di Scienze Lettere ed Arti", the University of Padua is organizing, during September 29–October 1, a symposium on "The experimental method in biology from the time of Antonio Vallisneri to the present". Further details can be obtained from the Organizing Committee, University, Via 8 Febbraio, Padova.

The Electronics Group of the Institute of Physics and the Physical Society announces that it is arranging a conference on "The Physics of Gas Discharge Devices", to be held at Leamington Spa during September 28–29. Further information can be obtained from the Administration Assistant, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

New Laboratories for the Division of Food Preservation, Commonwealth Scientific and Industrial Research Organization, will be opened at North Ryde, New South Wales, on September 18. A Food Science Conference will follow, during September 19–22. Further information can be obtained from the Chief, Division of Food Preservation, Commonwealth Scientific and Industrial Research Organization, P.O. Box 43, Ryde, N.S.W.

The Autumn Meeting of the Institute of Metals will be held in Brussels during September 18–22. The programme includes the Autumn Lecture, to be delivered by Baron Snoy et d'Oppuers on September 18 (who will speak on "The Non-Ferrous Metals Industry in a Unified Europe"), and sessions on deformation and fracture, on surface friction and lubrication in metal working and on corrosion. Further information can be obtained from the Institute of Metals, 17 Belgrave Square, London, S.W.1.

Erratum. In the issue of July 29, p. 439, the date of birth of Sir Frederick Gowland Hopkins was given as June 30, 1861; this should read June 20, 1861.

LONG ASHTON RESEARCH STATION, BRISTOL

THE staff of the University of Bristol Agricultural and Horticultural Station at Long Ashton includes 47 graduate scientific officers and now totals 225, approximately double the pre-war figure. The basic work of the Station on all aspects of fruit culture and fruit utilization has similarly expanded, and there has been, in addition, a rapid growth in biochemical studies related to plant nutrition, a development stimulated by the formation in 1952 of the Agricultural Research Council Unit of Plant Nutrition (Micronutrients), the members of which were assimilated into the Long Ashton staff in 1959.

These increases in staff and research programmes have necessitated new laboratories and ancillary services. A comprehensive building scheme for the Station was drawn up in 1949, and, although the proposed plans have not all been implemented, a substantial instalment of the programme has now been completed. On July 6, Sir William Slater, formerly secretary of the Agricultural Research Council, declared open the buildings that have been constructed during the past five years. Of these, the three principal are: (1) a range of experimental glasshouses with attached laboratories and special-purpose rooms; (2) a refectory/common room adaptable for use as a hall for lectures and meetings; (3) the Wallace Laboratory, named after Prof. T. Wallace who retired from the directorship in 1957 after having served the Station for thirty-eight years.

The Glasshouse/Laboratory block, a building of 43,000 sq. ft., has six separate glasshouses, each 72 × 34 ft. with a ridge-height of 24 ft., constructed of a suspended 'Aluminex' framework with side walls infilled with 2-ft. brickwork. The houses, from west to east, are used respectively for propagation, plant nutrition (two houses), pomology, plant pathology and entomology. Each glasshouse communicates by a short corridor to a header house 220 × 18 ft. The southern half of the header house, adjacent to the glasshouses, forms an open gallery; the northern half is divided to form laboratories, stores, cold rooms and special-purpose rooms with controlled environment. A set of rooms for work on radioactive tracers is segregated at the east end of the header house; the west end houses the services for soil and plant preparation.

The main boiler house, attached at the west of the glasshouse block, contains three oil-fired automatic-

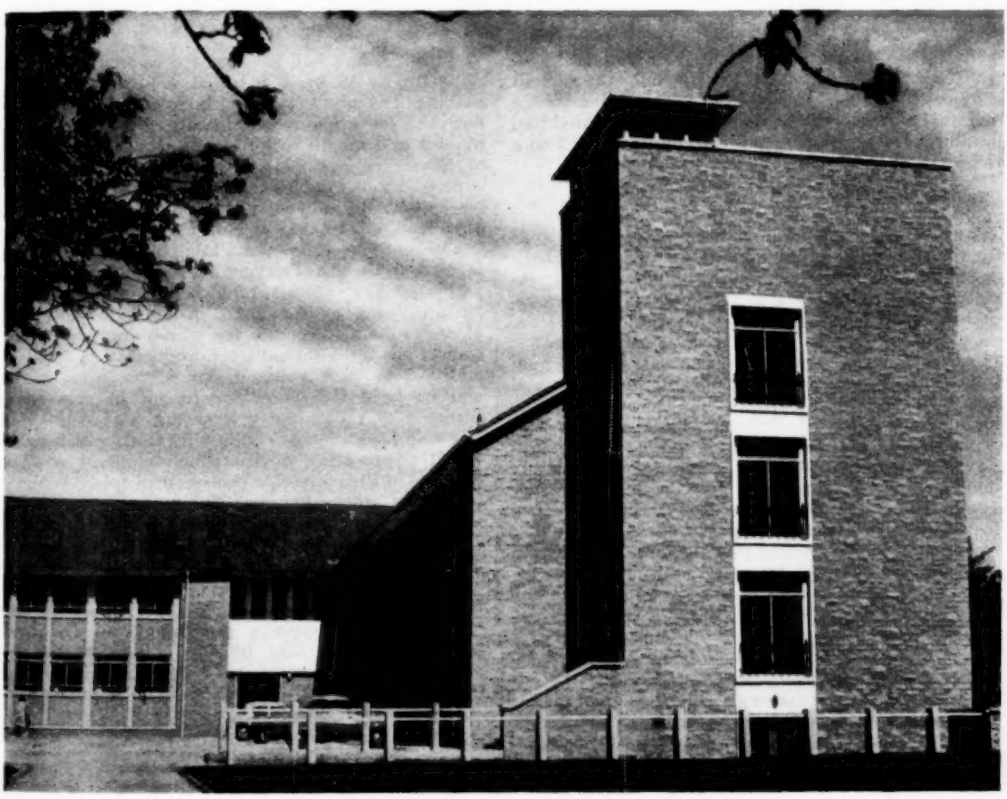

Fig. 1. The Wallace Laboratory of Long Ashton Research Station

ally controlled McNiel boilers, providing space heating and hot water for the glasshouses, the refectory and the Wallace Laboratory. Steam conveyed through underground conduits supplies heat exchangers for the production of hot water, which is passed through radiators, ceiling coils or hot-air circulators.

The Refectory/Common Room is a single-storyed building covering 5,300 sq. ft. It is faced with 'Reformite' reconstructed stone and has large french windows overlooking a pleasant expanse of the fruit plantations. The roof is of double-Roman tiles and the floor of polished Iroko wood blocks. The dining hall, 60 × 37 ft., connects through a folding partition to the common room 35 × 37 ft. With the partition open a hall 95 × 37 × 20 ft. high is provided. There is a well-appointed kitchen equipped with air extractors and refrigerated larder. The building is heated by a hot-air circulatory system linked to the main boiler house.

The Wallace Laboratory is a two-storyed, T-shaped building of 24,000 sq. ft. floor space, roofed and the walls faced in the same style as the refectory (Fig. 1). The entrance hall is panelled in apple-wood veneer cut from a tree of the cider variety 'Knotted Kernel' which formerly stood on the site. Some of its sister trees of the original cider orchard (planted in 1904) still flourish around the new laboratory. The western arm of the building houses the administration section on the ground-floor, and laboratories for analytical chemistry above. The southern limb of the ground-floor accommodates the pomology and plantations sections, with stores and organic chemistry on the first floor. The remainder of the ground-floor has laboratories for physical chemistry, statistics and willow research, with the section for nutrition of fruit plants above. There are extensive basement rooms for stores and heat-exchange equipment, with a tower at the southern end housing the water cisterns and capped by an observation platform for meteorological recording. The lofts house elaborate fume extractors and 'Plenum' heating equipment.

Among other buildings is one of 8,400 sq. ft. consisting of laboratories for biochemistry and plant physiology which was completed in 1956. During 1959–60, the director's room, the committee room and the library in the Barker Laboratory (the original laboratory building) were redecorated and re-furnished. Extensive improvements and additions have also been made to workshops, machinery sheds and roadways.

R. W. Marsh

THE BRITISH IRON AND STEEL RESEARCH ASSOCIATION

THE Sheffield Station of the British Iron and Steel Research Association, where open days were held on June 15 and 16, houses three of the Association's five main research divisions—the Steelmaking, Metallurgy (General), and Mechanical Working Divisions.

Steelmaking

The Steelmaking Division is responsible for research into all the modern processes involved in the conversion of iron into steel, and prominent among its present research projects are the control of electric-arc and open-hearth furnaces, continuous casting, and the use of oxygen in steelmaking operations.

The use of large electric-arc furnaces for the bulk production of steel is increasing and it is expected that, in 1965, these furnaces will account for one million tons more of Britain's total annual steel output than in 1960. Economy in the use of electrical power is becoming, therefore, a matter of increasing importance. The automatic power input controller (*Apic*), which has been developed recently by the Steelmaking Division for application to electric-arc furnaces, has already shown itself capable of achieving significant reductions in electricity consumption. Trials have been carried out satisfactorily on the Association's experimental furnace at Sheffield and on a 5-ton production furnace at Steel, Peech and Tozer, and further trials are now being carried out on another member's 30-ton furnace.

Continuous casting helps to achieve higher yields and greater uniformity of end product. A number of continuous casting plants has recently been installed or projected, including a twin-strand plant at the Abbey Works of the Steel Co. of Wales. This is based on the process developed by the Association in conjunction with the Continuous Casting Co. of Weybridge, and will be used for casting low-carbon slabs up to 48 in. × 8 in. in section. The Steelmaking Division's present investigations into this new process are directed mainly towards improved moulds and mould reciprocating cycles. Arising out of these investigations, a new design of mould has been evolved which enables three interconnected ingots to be cast simultaneously, these being afterwards separated and treated as individual castings.

During the past few years there has been a dramatic increase in the use of oxygen for steelmaking processes and the new vessels now being introduced into Britain, such as the *L.D.*, *Kaldo* and *Rotor*, will alone probably account for something like 14 per cent of the total annual steel production in 1965. In addition to pure refining duties, however, oxygen can be used for flame enrichment in open-hearth furnaces and also for pretreating hot metal. The Division is at present conducting trials of a new hot-metal pre-treating device which employs a simple reversal of the converter process principle. With this technique—termed spray refining—instead of passing oxidizing gases through molten metal, the metal is teemed through jets of oxidizing gas, thus breaking up the falling stream of metal and—by increasing the effective metal surface area—promoting rapid and efficient refining reactions.

The current research programme of the Steelmaking Division also includes investigations into control of open-hearth furnace combustion, the rapid desulphurization of electric steel and studies of subcutaneous segregates.

Metallurgy

The Metallurgy (General) Division is concerned with the physical, chemical and mechanical properties of iron and steel, and the effect on these properties

of variables in the manufacturing processes. The Division's activities are divided into the broad categories of physical properties, mechanical properties, metal treatment and metallurgical analysis.

Research into physical properties is at present focused on the magnetic characteristics of silicon iron. During 1960, electrical power to the value of eleven and a half million pounds was wasted as a result of losses in silicon iron transformer cores. A reduction of as little as 5 per cent in these losses would result in an annual saving of more than half a million pounds. The Division's investigations into the factors contributing to core losses have already shown that reductions of up to 15 per cent could be obtained.

A better understanding of the factors affecting the fatigue limit of steel would greatly improve the possibilities of achieving better dynamic mechanical properties in high-tensile steels. Also in the context of mechanical strength, it has been established that the presence of certain elements in mild steels affects their resistance to brittle fracture at low temperatures. The Division's work in connexion with the mechanical properties of steel is mainly concentrated on these problems, although research is also being conducted on the mechanism of fatigue and more economical methods of fatigue testing.

Metal treatment research, the third of the Metallurgy Division's spheres of activity, consists primarily of investigations into methods of degassing molten steel. The presence of certain gases in steel often has a significant effect on the qualities that are expected of it. Excess hydrogen, as a specific example, may lead to cracking and other undesirable flaws in the finished product. The Division is at present engaged in investigating two methods of hydrogen removal by degasification techniques. In the first of these a continuous vacuum degassing unit is used, molten metal being poured into an evacuated chamber, degassed at a pressure of about 1/1,000th atm. and discharged into air. In the second method—termed 'jet degassing'—the hydrogen is removed by blowing a neutral gas such as argon, nitrogen or helium on to the surface of the molten metal by means of a lance. This second technique has achieved a reduction of eight parts per million to one part per million in the hydrogen content of an eight-pound melt, and the possibility of using this method on a larger scale is being examined.

The increased throughput rates which have been made possible by the use of oxygen in steelmaking practice have thrown greater emphasis on the need for faster metallurgical analysis. In addition to developing faster and more accurate physico-chemical analytical procedures, the Division is also therefore investigating the application to steel works requirements of the newer physical techniques of direct reading and X-ray fluorescent spectroscopy and electron-probe micro-analysis.

Supplementary to its main research programme, the Metallurgy (General) Division also provides services to other research divisions and departments of the Association, to industry, to research organizations and to universities.

Mechanical Working

The Association's research into all the processes by which ingots are formed into wrought metal products is carried out by the Mechanical Working Division. This Division's research programme is therefore spread over a wide range of manufacturing processes which may be broadly classified by end-product—sheet and strip, forgings, rounds and sections, and wire.

Because of the superior quality of product obtainable, the use of continuous annealing for blackplate has been increasing in the tinplate industry during the past few years. However, conventional continuous annealing plants are very large and costly, and inflexible in operation. Investigations by the Division on an experimental annealing line at the Swansea laboratories have resulted in the development of a process which, by greatly reducing the pass length, makes possible the use of a more compact plant, with a considerable saving in costs. Products of a commercial quality have been obtained and plans are now in hand for the installation of a pilot production plant at a member's works.

Other features of the Division's research into sheet and strip manufacture include a new process for aluminizing steel strip, the electrolytic smoothing of blackplate, investigations into the prevention of scale formation on coiled strip, and three new in-line inspection techniques. Some fundamental work is also being conducted by the Association's Physics Department on tandem-mill automatic gauge control with the aid of a new analogue computer which has been installed recently at the Battersea laboratories of the Association.

In the manufacture of forgings the Division's main research project is the automatic programme control of forging presses and manipulators. Simple programmed forging operations have already been carried out, both more rapidly and more accurately than in manual operation, and more complicated schedules are now being formulated. Forging research has also resulted in the development of a new procedure for closing ingot cavities by upsetting, and a heat reflector arrangement which, by conserving the heat in the workpiece, has proved capable of achieving a 30 per cent reduction in heating requirements. In addition, the Plant Engineering and Energy Division is at present conducting investigations of batch type forge furnaces on behalf of the Mechanical Working Division.

The Division is also now working on a number of problems associated with wire manufacture—such as wire cooling, continuous wire inspection and control, and the welding of high-carbon steel wire—and the manufacture of rounds and sections.

Development and Information Services

The Sheffield Station also houses the Steel User Section of the Association's Development and Information Services. This Section employs the Association's research resources to assist individual companies in their use of the industry's products. The services which it provides range from answering simple technical inquiries to more complicated investigations into metallurgical and other production problems.

The remaining research divisions and departments of the British Iron and Steel Research Association are located, as in the case of the Sheffield Group, in the area of the country most appropriate to the work for which they are responsible. The Ironmaking Division, for example, has a pilot-plant laboratory at Normanby, near Middlesbrough on the north-east coast, and a blast-furnace laboratory at the Imperial

College of Science and Technology in South Kensington. The Plant Engineering and Energy Division is based at the Association's Battersea Research Station, as are the Physics, Chemistry and Operational Research Departments, and there is also a laboratory at Swansea, in the South Wales tinplate area, where some of the Mechanical Working Division's research into sheet and strip manufacture is conducted. The Association's headquarters are at 11 Park Lane, London, W.1.

THE INTERNATIONAL COUNCIL FOR BIRD PRESERVATION

THE seventh Conference of the European Continental Section of the International Council for Bird Preservation was held in Stavanger, Norway, during June 19–24, and was attended by representatives of the National Sections of Austria, Belgium, Denmark, France, Germany, the Netherlands, Norway, Sweden, Switzerland and the United Kingdom, together with representatives of the International Wildfowl Research Bureau, Conseil International de la Chasse, International Union of Biological Sciences, and the International Union for the Conservation of Nature and Natural Resources.

The destruction of sea birds by waste oil has become a permanent item on the agenda of all conferences of the International Council, and it was noted with great satisfaction that Poland had ratified the International Convention for the Pollution of the Sea by Oil, and that the U.S. Senate had voted 92–0 to ratify this convention. The adhesion of Poland to the convention is of particular importance to Scandinavian countries for it now only requires the support of the U.S.S.R. to enable steps to be taken to declare the whole of the Baltic a prohibited zone for discharge of oil. The area in the Baltic in which oil discharge is now permitted has resulted in heavy pollution, particularly on the coasts of Sweden, where it has caused such great destruction of the long-tailed duck (*Clangula hyemalis*) that it is feared the status of this species is endangered. The representatives of the Netherlands, where systematic counts of birds killed by waste oil are carried out throughout the year, reported that there were distinctly less than before, and this statement was also supported from Belgium.

A resolution was adopted welcoming the decision of the Inter-Governmental Maritime Consultative Organization to convene, in the spring of 1962, a further conference on oil pollution of the sea, and urging that priority be given to the complete avoidance, at the earliest possible date, of discharge of persistent oils into the sea, and to the immediate provision of adequate facilities for the disposal of oily wastes at refineries, ports of loading and ports of discharge. The resolution further urged National Sections to press their Governments to instruct their delegates to the Inter-Governmental Maritime Consultative Organization conference in 1962 to support action on these lines.

The prejudice against any bird with a hooked beak dies hard, and though some countries reported an increase in certain species it was generally agreed that birds of prey were still being destroyed on a large scale and many species had critically decreased. The need for education on the valuable part these birds play in the balance of Nature was expressed in a resolution which also recommended that if an examination is required to secure a shooting licence a knowledge of identification of birds of prey should be required, in order that protected species should be distinguished from those allowed to be shot.

The destruction of bird life as a result of the use of pesticides has reached such proportions as to cause grave concern all over Europe, and a resolution was passed requesting Governments to adopt legislation by which all pesticides must be officially tested not only with regard to their effect on noxious animals and plants but also on wild life in general before they are permitted to be sold. Further, that Governments be urged to encourage research on the use of specific pesticides and other methods which limit the destruction of wild life, and to forbid or place drastic restrictions on the use of general pesticides that are dangerous to wild life, wherever effective and less dangerous materials can be made available.

The great increase in herring gulls (*Larus argentatus*) and the harm these cause to other species, particularly in Germany and the Netherlands, has been a problem for many years. In the Netherlands methods of control have been carried out with great success, the number of breeding pairs in 1959 and 1960 being reduced to almost half that of 1954. One of the factors in the great increase of this species is the easy availability of food in winter, through fish offal at ports, rubbish dumps, etc. In Denmark there is no problem as all fish offal is collected and used for food in mink farms or for fertilizers.

The present policy of land drainage in certain European countries, whether it be economic or not, presents a serious threat to many species of water and wading birds, and two areas in particular were the subjects of special resolutions. The first, the Marismas of the Guadalquivir in Spain, is the richest area in western Europe for marshland birds, particularly as regards migrating and wintering species. The disappearance of these marshes would mean not only the loss of the main European breeding ground of several rare species but would also have irreparable consequences to the status of migratory marsh birds in many European countries. On June 1 an international delegation of representatives of the International Wildfowl Research Bureau, the International Council for Bird Preservation, and the International Union for the Conservation of Nature and Natural Resources, with the Consejo Superior de Investigaciones Cientificas of Spain was received by the Minister of Education in Madrid to consider the possibility of acquiring an area of the Marismas as a reserve and to establish a biological research station there.

The second area, the Baie de L'Aiguillon (Vendée) on the Atlantic coast of France, with its large expanse of mud flats, provides an exceptionally favourable resting place for migratory birds which breed in northern Europe and rest there on their spring and autumn migration. The project to drain this area, for agricultural purposes of doubtful value, in a

country where there is no lack of agricultural land is being strongly opposed by both French naturalists and sportsmen and their colleagues in other countries.

The proposals concerning the establishment of Council of Europe machinery for matters concerning nature conservancy and national parks were considered, and the prospect of a closer relationship between the Council of Europe and organizations for conservation of Nature was welcomed.

Among other subjects discussed were the traffic in European birds for cageing, bird protection in schools, the danger of the airgun and the increase of the starling (*Sturnus vulgaris*).

Prof. Sven Hörstadius (Sweden) was unanimously elected chairman of the European Continental Section in succession to Dr. Bøje Benzon (Denmark), and Dr. Gerth von Rokitansky (Austria), vice-chairman, in succession to Dr. G. A. Brouwer (Netherlands). Prof. S. O. Hörstadius, a former president of the International Union of Biological Sciences, was accorded an honorary degree by the University of Cambridge during the tercentenary celebrations of the Royal Society in 1960, and it may be apt to quote the Orator's citation on this occasion:

"We read in Lucretius these lines about the origin of animals: 'And many monsters also did the earth then try to create, springing up with strange face and limbs'. She did so to no purpose. But this man does the same to very good purpose. For beginning his studies from the egg in no mere proverbial sense, and using incredible penetration of mind and dexterity of hands alike, he has taken to pieces, down to their smallest components, the embryos of sea-urchins, worms and amphibia, and then re-assembled these into new animal organisms. Would anyone have believed before that two tissues cut from an embryonic newt combined could co-operate to form cartilage, and that, with the addition of a third, tooth would result? What wonderful metamorphoses, and advantageous indeed to men! For from this he has thrown light on the influence of part upon part and organ upon organ in the gradual construction of the adult animal, which could be of great value to the art of medicine. So we salute one who has at last set foot on the true way in an inquiry raised by Aristotle himself.

"But do not imagine he only examines and handles eggs. For he is an observer of the ways of birds, and in that most pleasant branch of study also has won world-wide fame.

"I present to you a professor of Uppsala, worthy heir of the studies of Linnæus of Uppsala—Sven Otto Hörstadius".

PHYLLIS BARCLAY-SMITH

IMMUNITY TO PROTOZOA

IN the age of Metchnikow and Ehrlich, the subject of immunity had not become divided into the separate compartments of bacteria, fungi, protozoa, etc., as it has to-day, and a broader view was possible at that time. A recent symposium, organized by the British Society for Immunology at the Middlesex Hospital during June 1–2, represented an attempt by the Society to reverse the trend and to bring workers of one specialized field (protozoology) into the classical milieu. Various aspects of immunity to protozoa were thus presented, and, to some extent the idea of the Society was successful, many interesting papers were given, and these provoked some discussion.

The subject of the symposium, "Immunity to Protozoa", was introduced by P. C. C. Garnham, who gave a short historical survey, then a summary of the histological basis of immunity and finally a review of acquired and innate immunity in the parasitic protozoa. In his opinion, the realm of innate or natural immunity offered at present the most promising opportunities for research. All degrees of resistance of the host to the organism are found, which may be influenced by various internal factors like unsuitable blood components (later ably described by A. C. Allison in regard to the inhibitory action of sickle-cell hæmoglobin and glucose-6-dehydrogenase deficiency on *Plasmodium falciparum*), by external factors like removal of the spleen, and lastly by the site occupied by the parasite in relation to its host: immunity thus may be unable to touch the parasite when it is safely enclosed within a parenchyma cell of the liver, yet immunity will kill rapidly when it lies in an exposed position in the blood stream.

The symposium was honoured by the presence of Edmond Sergent, who came from Algiers to give a lucid account of premunition, a subject which has occupied him for the past 60 years and which he here crystallized into 26 precise definitions. Premunition is the basis of the subject, and yet has been much misunderstood; it was useful to have it described by the *Maître* himself. On the other hand, premunition is not the only form of immunity, and A. Corradetti described examples of true residual immunity in rats which had recovered from *Plasmodium berghei* and *Trypanosoma lewisi* infections respectively; in some cases, nothing could break down this resistance, and he clearly showed that the protozoa had been completely eliminated by the immunity response, while the rats proved to be immune for life.

Much of the original work on the cellular basis of immunity was carried out by W. H. Taliaferro, by his wife Lucy, and his collaborator, H. Mulligan. All these workers were present at the symposium, and the subject was introduced by W. H. Taliaferro, who described briefly the lymphoid-macrophage system and then went on to more recent work of a biochemical nature. This indicated that under the influence of the growth-inhibitory antibody, 'ablastin', nucleic acid synthesis in *T. lewisi* comes to a stop, although protein synthesis may continue at a low level. He also considered the phenomenon of 'avidity' or antigen-binding capacity. His explanation of the development of immunity in *T. lewisi* infections by the successive appearance of several different types of antibody was challenged by W. E. Ormerod, who suggested that the disappearance of young and dividing forms (the earliest of which inhabit the protected environment of the kidney capillaries) was not due to 'ablastin' but to an ordinary type of antibody which had a selective action on the young forms.

The subject of immunity cannot properly be considered apart from the nature of the damage inflicted

by the parasite on the host, in other words, the mechanism of pathogenicity. B. G. Maegraith illustrated this by reference to non-invasive strains of parasites, which later change their habits and attack the tissue so that an increasing degree of virulence is attained. In *Entamoeba histolytica*, the special enzyme production of an amoeba determined its pathogenic potential. The actual cause of injury was thought, in the case of malaria, to be a soluble toxin which acted on the mitochondria of cells, destroyed their membranes, and brought respiration to an end. The chemical composition of this agent is still not precisely known, but it appears to be non-specific. This work seems to be on the brink of important new discoveries.

The symposium then continued by examining the phenomenon of immunity in: (*a*) blood infections; (*b*) tissue infections; (*c*) infections of the skin and mucous membranes, including the intestinal tract. The last-mentioned group stood out from the rest because some localization of immunity was often apparent.

Immunity in malaria was discussed by Avivah Zuckerman. *In vitro*, macrophages exposed to malarious chicken blood, in the presence of hyper-immune homologous serum, phagocytosed numerous normal as well as parasitized erythrocytes. Furthermore, in a series of simian, rodent and avian malarias, more anæmia occurred than could be attributed to destruction of red cells by emerging parasites. An autoantibody was postulated to explain these observations, and Coombs' tests were positive in two rodent malarias during the period of excessive anæmia. However, since positive antiglobulin tests were also observed in other anæmic states from which parasites were absent, the autoantibody hypothesis remains moot.

L. J. Bruce-Chwatt gave an interesting account of the variability of immunity in malaria inherited by an infant from its mother, and which might be derived either via the placenta (particularly in human beings) or via the milk (in rodents). There was no doubt of its existence in the hyperendemic regions of tropical Africa, and it formed a valuable barrier to infection in the earliest days of life, though the effect quickly wears off. Transplacental immunity is probably the explanation of the unexpectedly low rates of occurrence of parasites in infancy. The exact mechanism is still unknown, and it was suggested that the subject might be investigated further in monkeys, where the placental structure was not unlike that of the human.

Immunity in piroplasmosis was considered by R. Riek, and in a written communication by S. F. Barnett. The former described babesiosis in Australian cattle, demonstrating once more the peculiar tolerance of calves to *Babesia* infections in contrast to the susceptibility of the adults. He made some interesting references to the pathogenic effect of this organism, when present in a high density, on the invertebrate host, a subject which had been little discussed, perhaps because the arthropod is normally immune to any harmful effects of the protozoon which it carries. S. F. Barnett divided the *Theileria* species in cattle into three immunological groups: (1) *T. mutans*; (2) *T. annulata*; (3) *T. parva* (which includes *lawrencei* and *bovis* in addition to *parva*). The last-mentioned group behaves differently because it produces a sterile residual immunity while the other two are followed by premunition. This worker stated that the endoglobular parasites and the intra-lymphocytic schizonts gave rise to distinct antibodies, and that after recovery from an endoglobular infection, the animal was still susceptible to the tissue forms, which were finally destroyed by lytic antibodies.

Immunity in trypanosomiasis was discussed in four papers. B. Weitz described his recent researches on exo-antigens in infections with the African trypanosomes. K. N. Brown analysed the antigens of *T. rhodesiense*, a highly labile organism which resorts to many changes in antigenicity, though reverting to the parent type after passage through *Glossina*. Two groups of antigens can be distinguished chemically by starch-gel electrophoresis and by ultracentrifugation; one type is predominantly protein, the other is of unknown composition. This chemical approach has scarcely gone far enough yet for conclusions or principles to be drawn, but it offers promise for the future, especially when combined with the agar techniques using double diffusion of separating strains. A paper by M. A. Soltys and C. S. Folkers was presented by the latter and dealt with the effect of immunity on chemotherapy and chemoprophylaxis of the African trypanosomes of veterinary importance.

Two papers were given on toxoplasmosis. The first, by J. D. Fulton, gave a useful summary of the immunity reactions in this disease, and included details of the author's new agglutination test, using washed organisms from mouse peritoneal fluid. The results of this test corresponded closely with those of the Sabin–Feldman reaction, were constant, easy to read, and beset with none of the difficulties of the dye test. C. P. Beattie discussed the prevalence of the infection throughout the world in man, in other mammals and birds; but *Toxoplasma gondii* is a well-adapted parasite which rarely causes much pathogenicity to its host, either because most strains are of naturally low virulence or because of some special insusceptibility of the host. Youth, concomitant illness, and physical stress are factors which predispose to multiplication of the organism. Although several antibodies have been shown to exist in chronic infections, it is strange that none is particularly adequate in destroying the organism, perhaps because it eventually becomes enclosed in a cyst with an impermeable wall. But man has a natural resistance to the infection, which may be of the nature of premunition, the organism persisting in a latent form for years, and resembling in this and other aspects the tubercle bacillus.

Saul Adler described immunity in leishmaniasis, and discussed chiefly the reaction of man to the various cutaneous forms of *Leishmania*. He made a plea for the recognition and separation of these organisms into sub-species, of which there were two in the Old World and at least four in Latin America, causing the classical espundia in Brazil, uta in Peru, chiclero's disease in Central America and a curious form in Venezuela. These were distinct clinical entities, had special sandfly vectors, particular animal reservoirs, characteristic response to drugs and specific antibody reactions by which each form could be distinguished readily. He directed attention to the paradox of a protozoon making its home in cells of the lymphoid-macrophage system, the system which, *par excellence*, protects the host from protozoal infections. Nevertheless the host wins, and a persistent sterile immunity is the result.

Adler's paper formed a good introduction to the last part of the symposium, which concerned parasites localized in the skin or mucous membranes. The immunity reactions of this group have a peculiarly focal restriction, in contrast to the ordinary antibody response when the infection is generalized; but the difference disappears if these localized parasites are introduced artificially into the blood stream of the host, when a typical antibody reaction is the result. Muriel Robertson showed, however, that such antibodies, produced in response to *Trichomonas foetus*, were unable to reach the flagellates in their protected sites in the uterine wall; the production of antibody is a sequence of the presentation of antigen to the appropriate host cells; in trichomoniasis of cattle the antibody had a very limited value in destroying the infection.

C. Horton-Smith, P. L. Long, A. E. Pierce and M. E. Rose also examined the question of local immunity in coccidiosis and showed that a fowl which had recovered from an infection of *Eimeria tenella* in one cæcum was nevertheless immune in the other. These workers demonstrated that different stages of the life-cycle of coccidian parasites had their specific immunizing values. R. Augustin and A. P. Ridges described similar work on *E. meleagrimitis* of turkeys.

In the early days, immunologists were much concerned in the practical aspects of cure or prophylaxis of disease: two papers in the present symposium show that this subject is still of interest to-day. S. Cohen and I. A. McGregor gave a most interesting account of the use of gamma-globulin in the cure of malaria. They collected sera from hyperimmune adults living in the Gambia and extracted the gamma-globulin component; this was then given in large doses to young children who were suffering from acute infections of the disease. Within 4 days, parasitæmia fell and by the ninth-day parasites were undetectable in 8 of the 12 cases studied. These investigations were accompanied by work on the turnover of gamma-globulin, on the lessening of hypergamma-globulinæmia as the result of specific therapy, etc., all of which observations tend to indicate that humoral immunity may be more important in malaria than was originally thought. The antibody appears to be contained in the 7-*S* fraction and not in the macroglobulin. P. E. C. Manson Bahr sent an important communication on a big field experiment he is carrying out in Kenya, in which he has immunized a population which is exposed to epidemic kala-azar with an avirulent ground squirrel strain of the organism. Preliminary work had shown that volunteers could be protected in this way.

The papers are being published in book form, and this should provide a modern review of many important aspects of the subject. P. C. C. GARNHAM

THE MUSEUMS ASSOCIATION

IN the ideal environment of the City of Plymouth and its museums the sixty-seventh annual conference of the Museums Association was held during June 19–23, 1961.

The conference was officially welcomed by the Lord Mayor and this was followed by the presidential address by Dr. D. B. Harden (London Museum). In an interesting historical survey of the continual efforts of the Museums Association since 1945 to obtain Government grants for the provincial museums —and especially the smaller ones—he felt that there had been a certain amount of progress, for at last the Standing Commission for Museums and Art Galleries had been instructed to prepare a report. This was awaited with interest, though, meanwhile, the Association and museums throughout Britain were grateful to the Carnegie United Kingdom Trust for its continual help and advice. Reference was also made to the formation of the Area Councils and especially to the progress made by that in the south-west.

Sir Hugh Casson opened the discussion on display in museums, and in a provocative and entertaining address said that a lively imagination was necessary both in new buildings and in the adaptation of the older ones. He stressed that the building and display must be shared by the curator and the architect, and that it was essential to establish character and personality in a museum. A museum must be an active place—a lively cultural centre to arouse the sense of the curious.

Dr. D. A. Allan felt that the ideal display of natural history objects should include the clinical and the artistic. He also stressed the value of genuine objects, and said that it was important to achieve liveliness and realism. Large dioramas were not in the fashion at the present day. Mr. J. W. Y. Higgs (University of Oxford) dealt chiefly with folk material, and detailed the value of comparative displays—doing the same thing in different ways. He also referred to the need for more folk parks in Britain. Mr. Norman Cook (Guildhall Museum) stated that it was necessary to serve the public at different levels. He also felt that not all archæological exhibits should be treated as works of art, for it was not the curators' task to make everything look beautiful. As chairman of this session, Sir Frank Francis (British Museum) felt that there was no single solution to the problem of display for museums were both for the connoisseur and the ordinary visitor. The future may lie in some form of double exhibition.

A whole day was devoted to the many problems of conservation. Dr. P. Coremans (Brussels) opened the discussion, and said that great changes in the treatment of paintings was due to the scientific interest now taken in this subject. He described methods whereby it was possible to distinguish the layers of dirt, re-paint, varnish and the glazes which were an integral part of the picture itself. The extreme differences of opinion regarding restoration were also discussed. Mr. Norman Bronmelle (Victoria and Albert Museum) suggested a scheme for making modern scientific resources available to provincial museums, and Mr. H. Schubart (Bristol) spoke about the training of picture restorers. An art school basis was probably the best, but the creative impulse of the restorer must be directed entirely to conservation so that the welfare of the object became second nature.

An afternoon session was devoted to a symposium on modern developments of conservation in museums as opposed to art galleries. Dr. A. E. Werner

(Research Laboratory, British Museum) stressed the wider concept of conservation which involved successively preventive treatment, diagnosis of the causes of decay, research in the evaluation of new synthetic materials and scientific examination of the object before attempting treatment. Mr. H. W. M. Hodges (Institute of Archæology) said that at present there were only two academic training institutions in the world for conservators—in London and in New York. He detailed the training given in London and emphasized that conservation must be based on scientific knowledge. Mr. A. E. Rixon (British Museum (Natural History)) gave details of useful techniques for dealing with the preservation of fossils.

At the annual general meeting, Dr. Mary Woodall (Birmingham) was elected president for the ensuing year, and an invitation to hold the conference in Brussels in 1962 was accepted with gratitude.

Social events during the week included a civic reception and an annual dinner. At the end of the week organized tours to many of the museums and beauty spots in Devon and North Cornwall brought a memorable conference to a close.

F. S. Wallis

DIGITAL COMPUTERS, THEIR USE AND CONTROL

A SYMPOSIUM on "Digital Computers, Their Use and Control" was organized by the Mathematics Department of Queen's College, University of St. Andrews, in collaboration with Standard Telephones and Cables, Ltd., during May 29–June 2. Standard Telephones and Cables, Ltd., provided the use of a *Stantec Zebra* computer for the whole course.

The first day was devoted to talks of a general nature to give a general appreciation of the use of computers. After an address of welcome by the Master of Queen's College, Prof. A. A. Matheson, Dr. R. J. Ord-Smith of Standard Telephones and Cables, Ltd., spoke on computer applications and this was followed by an address by Mr. W. A. Donaldson of Rolls Royce, Glasgow, entitled "Computers in Industry". The morning session was completed by a description of a transportation problem and its solution by Mr. W. A. E. Pillow of Standard Telephones and Cables, Ltd.

In the afternoon the symposium split into two sections. Those who wished to attend the whole course were given a demonstration of *Zebra* programmes and the remainder were given a brief introduction to programming.

The first day was attended by about eighty people of whom about thirty-five were from local industry and commerce and the remainder from the University. On subsequent days, about fifty people attended of whom ten were from outside the University.

The lectures on the remaining days were given largely by Dr. R. J. Ord-Smith and Mr. A. Y. Cooper of Standard Telephones and Cables, Ltd. They covered a comprehensive use of the *Zebra* simple code and an introduction to normal code. A lecture on "The Computer in the University" was given by Dr. A. J. Cole of Queen's College. By the end of the week, all delegates had written and run several programmes.

The members of Queen's College staff are grateful to Standard Telephones and Cables, Ltd., for providing such excellent facilities and in particular for the continued loan of a *Zebra* computer, which, in the week subsequent to the course had been in continual use for 8 hr. a day.

A. J. Cole

J. Iball

A NEW APPROACH TO BIOLOGY TEACHING

A CONFERENCE, proposed and financed by the Gulbenkian Foundation, was held in the University of Birmingham during April 10–12, to discuss the biology syllabus for sixth forms, proposed by the Biology Panel of the Gulbenkian Enquiry 1959*. The conference was attended by seventy-five schoolteachers and several lecturers from teacher-training colleges. The majority of the members came from the West Midlands. Several topics were selected from the syllabus and a lecture was given on each.

During his introductory lecture Prof. O. E. Lowenstein pointed out that the University of Birmingham will be offering a new type of biological training in the near future. This will consist of an honours B.Sc. degree in biological sciences. Undergraduates will take a variety of biological courses which will cut across the traditional subject-boundaries and be in closer accord with the trends in modern biology in pure and applied research, in industry, in agriculture, etc. Various subject-combinations will be possible.

* Copies can be obtained from the Registrar, The University, Birmingham 15.

It is anticipated that fewer students will complete courses of the traditional type leading to a degree in zoology or botany, although it will still be possible to specialize in selected branches of biology, for example, systematic botany, entomology, etc. It is difficult to predict whether other universities will adopt similar schemes; but the wind of change is blowing in this direction and during recent years in many university departments, even within the traditional courses of zoology and botany, the subject-matter has changed dramatically. Already in Birmingham the preliminary (= intermediate) course in biology has ceased to be virtually separate courses in botany and zoology and is now a closely integrated course following the lines recommended in the Gulbenkian Report.

It is against such a background of a rapidly evolving subject that this new approach to biology in sixth forms is presented.

During discussion there emerged considerable agreement on the need for a new approach, both to teaching and to examining, of the type put forward in the Gulbenkian syllabus, although it was recog-

nized that there would be various difficulties in implementing the recommendations of the report. It was suggested that a first step in the right direction would be to persuade the Northern Universities Joint Matriculation Board to set up a pilot scheme to give the new syllabus a 'field trial'. The chairman was instructed to write to the Board and make this proposal. He was also asked to produce a memorandum listing a number of specific difficulties involved in teaching biology and to circulate the memorandum to the Institute of Biology, local education authorities and other bodies who might be interested.

The points which emerged during the discussion were as follows: (1) Lack of exclusive use of biology laboratories by many teachers. This makes adequate preparation for practical work very difficult or impossible. It also makes difficult the keeping of cultures, aquaria, etc. (2) Lack of time for collection of material and preparation for practical work. (3) Lack of technical assistance. (4) Lack of preparation rooms and storage facilities. (5) Lack of transport for collecting and field work. (6) Inadequate grants for the purchase of material, books and equipment, such as physiological apparatus, stereoscopic microscopes, phase-contrast microscopes, aquaria, cages, incubators, ovens, refrigerators, deep freezers, etc. These items include essential basic equipment for the proper study of biology. (7) It was recognized that staffing and salary problems might arise if zoology and botany were replaced by a single biology.

It is relevant to all these items that the conference was agreed that teachers of biology require more time for the collection of material and the preparation for practical work than teachers of most, if not all, other subjects. Many teachers at the present time have to 'make do' in the face of considerable difficulties. The adoption of a syllabus like that proposed would increase these difficulties. The problems involved in the day-to-day teaching of biology in schools require objective study. There is no logical foundation for the assumption that every science subject requires the same number of teaching-hours for its presentation.

L. H. FINLAYSON

SCIENCE AND EDUCATION: THE TEACHERS' POINT OF VIEW

A POLICY Statement, "Science and Education"*, issued by the Science Masters' Association and the Association of Women Science Teachers, revises the preliminary statement published by the Science Masters' Association in 1957. The Statement is accompanied by separate syllabuses on chemistry, physics and biology for grammar schools compiled by panels of the Science and Education Committee for these subjects and amended in the light of comments received. The Policy Statement now issued concerns mainly grammar schools and represents the first part of the promised report. The second part will include a scheme for the general study of science in the sixth form, and two other panels are concerned with the training of teachers and with new experimental work necessary for teaching modern physical science.

The present 'scientific illiteracy' is attributed partly to a lack of factual knowledge but much more to a lack of understanding of the basic nature and aims of science. Science should be recognized and taught as a major human activity which explores the realm of human experience, maps it methodically but also imaginatively, and, by disciplined speculation, creates a coherent system of knowledge. As a human quest for truth, it is much concerned with basic values and is indeed an active humanity, and it follows that schools have the duty of presenting science as part of our cultural and humanistic heritage, to be taught in harmony with, not in opposition to, the various arts subjects which alone have hitherto been called humanities. The Committee recognizes that an adequate supply of scientists and technologists is needed and believes that its recommendations will help to meet that need, but it stresses the cultural aim because science has not yet been given its proper place in general education.

It may be apposite in these pages to comment how Sir Richard Gregory, with his passionate belief in the cultural value of science, would have welcomed such a statement from such a source. To ensure that science is a 'core' subject in grammar schools, as English and mathematics now are and as classics used to be, the Committee recommends that *all* pupils should follow a balanced course of science subjects up to the end of their fifth form year, and this course should lie within the range of a group of natural sciences, each of which is concerned with a limited aspect of human experience, though it is essential that this formal course, mainly involving the sciences of biology, chemistry and physics, with some astronomy and geology, if possible, should help pupils to appreciate the wider range of science as a whole. There should be no division into science specialists and arts specialists until beyond this level, though the Committee recommends that subject specialization should be retained in the sixth form but with a reduction of the detailed factual content of the Advanced Level syllabuses for the General Certificate of Education.

Such a reduction should release time for the broader course of science which the Committee recommends should be studied by all pupils in the sixth form, a course which would enable pupils to attain the scientific literacy described as 'numeracy' in the Crowther Report. The implications of these three general recommendations are outlined in the remainder of the Statement which describes, first, the introductory phase covering approximately the first two years of a grammar school course; secondly, an intermediate plan, usually three years up to about sixteen, when the Ordinary Level examination for the General Certificate of Education may be taken; and, thirdly, an advanced (sixth form) plan, offering a course indicated for all students and a course for the science specialists. These are further amplified in the three accompanying sections dealing, respectively, with chemistry, biology and physics, which set forth a syllabus which might appropriately meet

*Science Masters' Association and the Association of Women Science Teachers. *Science and Education: A Policy Statement*. Pp. 12. 1*s*. net. *Chemistry for Grammar Schools*. Pp. 13. 2*s*. net. *Biology for Grammar Schools*. Pp. 24. 2*s*. net. *Physics for Grammar Schools*. Pp. 30. 2*s*. net. The Sections of Part 1 of the Science and Education Report to be read in conjunction with the Policy Statement. (London: John Murray (Publishers), Ltd., 1961.)

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the needs of all three phases of the proposed policy. The syllabuses seem well designed to meet some of the criticisms which have been made of the outlook of science graduates themselves, and the Statement and its accompanying detailed sections demonstrate that the two Associations are tackling the revision of syllabuses imaginatively as well as energetically. They are to be commended not simply to the attention of science teachers themselves but at least as much to scientists and the professional associations, as well as to the universities, for the recommendations can scarcely achieve their full effort without full co-operation by the universities and professional bodies. Moreover, considering the steps already taken by the National Science Foundation in the United States, and in view of the serious shortage of science teachers both the Minister of Education and the Minister for Science should be considering the wider implications, and the steps that will be required at a higher level if the recommendations are to be adopted not merely in grammar schools but also elsewhere.

ATOMIC RADIATIONS AND LIVING ORGANISMS

SINCE 1955 various committees appointed by the U.S. National Academy of Sciences—National Research Council have been conducting a continuing appraisal of the effects of atomic radiations on living organisms. Various reports have come from sub-committees of the Committee on Pathologic Effects, and differ considerably in their approach and content[1].

The most generally valuable of the recent reports is that on *Inhaled Radioactive Particles* (ref. 1*a*). It contains, or provides references to, basic factual information on the nature of these particles, and the physiological and pathological mechanisms involved in their deposition in and translocation from the respiratory tract. The formidable complexity of the problems involved is clear.

Different processes are responsible for the retention of particles of different sizes and densities, and in practical situations the amount of radioactivity in a given particle is independent of the nature of the radioactivity and of the size of the particle. The rate of loss of radioactivity from particles deposited in the respiratory tract is not simply related to 'solubility' in body fluids. The problems of assessing radiation 'dose' to the tissue around a radioactive particle go to the root of what is meant by tissue dose, a concept which still awaits a fruitful synthesis of physical chemistry, cell biology and pathology, at least in relation to carcinogenesis. Although miners of radioactive ores have suffered a gross excess of cancer of the lung for several centuries, the relative importance of the different factors which may be concerned, including those non-radiative, is quite obscure.

Another report (ref. 1*b*) entitled *Long-Term Effects of Ionizing Radiations from External Sources*, is more discursive and covers the same ground as many fairly recent reviews: life-shortening, tumour production, cataract, fertility and ageing. Seven of the eleven sub-committee members come from the same university, five from the same department, and it is not unfair to say that this common background permeates the theoretical framework or point of view. The implication that ovarian damage in the adult human female is not irreversible (p. 27) would not be generally acceptable. The definition of threshold dose as a description of the possible response of a single animal (p. 2) seems likely to add further confusion to the existing conceptual muddle.

A short report (ref. 1*c*) on the *Effects of Ionizing Radiation on the Human Haematopoietic System* has also been issued, but much that is new is an expression of opinion, for example, on the proper clinical treatment for an individual exposed to a dose in the acutely lethal range. Routine blood counts at periodic intervals in occupationally exposed persons are "wasteful and unproductive in detecting low-dose radiation effects". When decisions are to be made involving exposure of populations, *society* must weigh the benefits against the potential health hazard (my italics).

R. H. Mole

[1] National Academy of Sciences—National Research Council. (*a*) Publication No. 848: *Effects of Inhaled Radioactive Particles:* Report of the Subcommittee on Inhalation Hazards, Committee on Pathologic Effects of Atomic Radiation. Pp. vi+78. (*b*) Publication No. 849: Report of the Subcommittee on *Long-Term Effects of Ionizing Radiations from External Sources*, of the Committee of Pathologic Effects of Atomic Radiation. Pp. v+82. (*c*) Publication No. 875: *Effects of Ionizing Radiation on the Human Hemapoietic System:* Report of the Subcommittee on Hematologic Effects, Committee on Pathological Effects of Atomic Radiation. Pp. v+14. (Washington, D.C.: National Academy of Sciences—National Research Council, 1961.)

SLOW DECOMPOSITION OF EXPLOSIVE CRYSTALS AND THEIR DAMAGE BY FISSION FRAGMENTS

By Dr. F. P. BOWDEN, C.B.E., F.R.S., and H. M. MONTAGU-POLLOCK
Physics and Chemistry of Solids, Cavendish Laboratory, Cambridge

Thermal Decomposition

WITH many explosive solids the decomposition may begin slowly before it passes over into rapid explosion or detonation. The onset of explosions is usually governed by thermal considerations: if the rate of heat liberated by the slow chemical decomposition of the crystal is greater than that of heat lost by conduction, the reaction will accelerate to explosion[1]. The mechanism of slow decomposition is therefore of considerable importance in determining the explosive process. We have in this Laboratory been using electron microscopy and electron diffraction to study the early stages of the slow decomposition[2–5].

Similar methods have also been applied to non-explosive crystals[6,7]. In the earlier work on explosive crystals, replica techniques or the scanning electron microscope were used. More recently we have been using direct transmission electron microscopy—which reveals more detail—to study both thermal decomposition and the decomposition produced by fission fragments. Silver cyanamide (Ag_2CN_2) was the crystal chosen for this work. Although it is explosive it was found to be stable in electron beams of moderate intensity, and the crystals could be grown thin enough (about 100 molecules thick) to be observed in transmission. The main products of decomposition at low temperatures are silver, carbon and gaseous nitrogen. The formation, movement and aggregation of the liberated silver can be followed in some detail.

The crystals were heated in a furnace *in vacuo* under controlled conditions at temperatures ranging from 150° to 350° C. and then placed in the microscope for observation. They were supported on carbon films lying on the specimen grids. Diffraction patterns of selected areas were taken in order to identify undecomposed and decomposed material. As well as qualitative information on the decomposition process, rough measurements of rates of decomposition were obtained from estimates of the volume of silver liberated, with the help of metal shadowing.

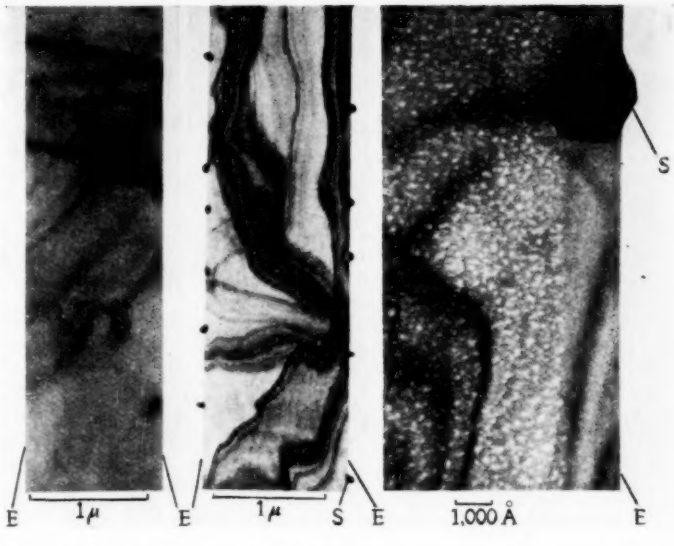


Fig. 1*A*. Symbols: *E*, true edge of crystal; *S*, silver aggregate. Undecomposed silver cyanamide crystal, about 200 Å. thick. The only visible details are the Bragg extinction contours, arising from slight elastic distortion of the crystal

Fig. 1*B*. Crystal in the first stage of thermal decomposition. A layer of the crystal extending from the surface to a depth of about 15 Å. has decomposed. The silver produced has diffused over the crystal surface and formed a few opaque specks at the the edges

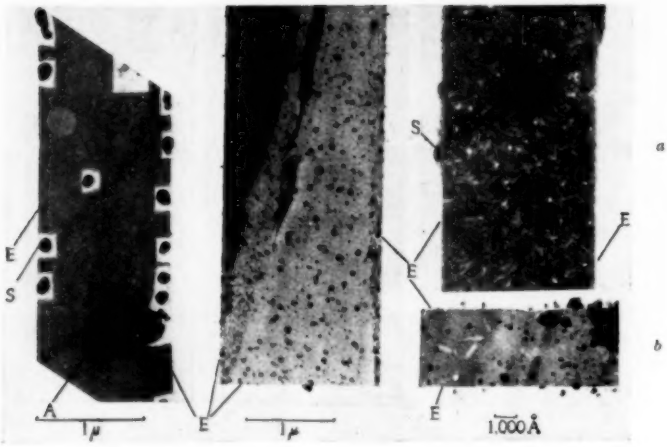
Fig. 1*C*. Crystal in the second stage of thermal decomposition. Surface mobility of the silver is again apparent, with no evidence for nucleation of decomposition at the silver specks. Note the sharply bounded region of surface mottling

Fig. 1*A* shows an undecomposed crystal of silver cyanamide. Such crystals gave diffraction patterns consistent with the (100) orientation of the structure of silver cyanamide as determined by X-ray methods: this work will be described elsewhere and gives a monoclinic unit cell containing four molecules, with $a = 7{\cdot}26$, $b = 5{\cdot}92$ and $c = 6{\cdot}61$ Å., and with $\beta = 102° 20'$. The space group is $P2_1/c$.

Fig. 1*B* shows a crystal which has been heated for 2 min. at 200° C. A number of opaque specks can be seen at the edge of the crystal. Electron diffraction measurements show that these are single crystals of silver of random orientation. It would seem that the liberated silver is highly mobile and can migrate over the surface to sites of high surface energy on the crystal. These are frequently at the edge but may be elsewhere. These silver specks normally project from the crystal surface and, as they grow, develop more or less well-defined crystallographic faces (Fig. 1*C*); occasionally whiskers are seen. Nucleation, in the sense of decomposition spreading outwards from certain points, is never observed. Instead, the silver is liberated by an overall surface decomposition, before diffusing to the aggregation sites; this was deduced from plots of volume of silver per unit surface area of crystals against time at various temperatures, and confirmed by the observation that thermal decomposition was inhibited almost entirely if the crystals had previously been coated with a thin layer of carbon, evaporated on to the surface *in vacuo*. If the heating is continued for a sufficient length of time, the crystal is completely decomposed into crystalline silver and an amorphous layer which gives a diffraction pattern similar to that given by amorphous carbon (the possibility that some of this amorphous material may be formed by the polymerization of cyanogen[8] is not ruled out).

At temperatures below 230° C. the decomposition is found to take place in two stages. The first stage involves a progressive thinning of the crystal: the rate of this is of course dependent on temperature, and at 170° C., for example, it is about 0·7 Å./min. When the crystal has grown thinner by about 20 Å. (5 atomic layers), a second and much slower stage of the decomposition sets in. The thinning of the crystal continues at this stage until the crystal is completely decomposed, but the rate is about one-tenth of that observed in the first stage, for example, about 0·07 Å./min. at 170° C. It is possible that amorphous carbon formed during the first stage is responsible for the slowing down of the decomposition at the beginning of the second stage. As the decomposition proceeds, a mottling becomes visible, suggesting that the crystal surface is developing pits about 130 Å. in diameter, and consistent with the random surface decomposition process. The crystal in Fig. 1*C* is at this stage, and shows a characteristic often seen: the central part of the crystal is much more decomposed than the parts near the edges, where the mottling has not yet begun. The lines dividing these regions are perfectly straight, and there is evidence from other experiments that they may be associated with steps on the surface of the original undecomposed crystals.

If the crystals are heated in the presence of oxygen, an additional type of decomposition is observed:



A B C

Fig. 2A. Crystal heated in the presence of oxygen. Additional decomposition takes the form of holes of crystallographic shape, starting around each silver speck and continuing to grow when isolated from it. Silver liberated by the growth of the holes has diffused over the crystal surface to aggregate at A

Fig. 2B. Crystal partially decomposed by the combined effects of heat and electron irradiation. A large number of specks of silver has appeared

Fig. 2C. (a) Crystal irradiated with uranium fission fragments (neutron flux density of $4{\cdot}3 \times 10^{12}$ n.v.t.). Each light area is a tunnel where material has been decomposed by a fragment: the dark areas are aggregated silver. (b) Crystal similarly irradiated (at a lower neutron flux density, $7{\cdot}2 \times 10^{12}$ n.v.t.)

the silver specks formed during the first and second stages cause the crystal to decompose through its whole thickness in their immediate neighbourhood, so that a hole appears around each speck (Fig. 2A). These holes rapidly take on crystallographic shapes and grow at a constant rate. The holes first become visible during the second stage, when the rate of growth of a silver speck is overtaken by the rate of growth of a hole. The liberated silver tends to diffuse over the crystal surface as before, rather than enlarge the new isolated silver specks. It aggregates at one or two specific sites.

If the electron beam intensity in the microscope is raised above a critical value, decomposition of the crystal occurs in the microscope. Under these conditions the liberated silver forms a large number of small specks distributed over the surface of the crystal (Fig. 2B). A similar distribution of the silver is obtained if the crystal is heated in a furnace to a higher temperature ($> 300°$ C.). If the beam intensity is raised still higher the crystal explodes giving silver and amorphous carbon. Heating to a high temperature will produce a similar explosion. If a cold stage is used in the microscope, the decomposition in the electron beam is inhibited. This behaviour is consistent with the view that the main effect of the intense electron beam is to heat the crystal to a high temperature.

Decomposition by Fission Fragments

Earlier experiments have shown that (with certain exceptions such as nitrogen iodide) most explosive crystals do not explode when irradiated with a moderate intensity of neutrons or fission fragments[1]. Important changes are, however, produced and the explosive may be rendered more sensitive to subsequent thermal decomposition.

A study has been made in the microscope of the damage and of the tracks produced in silver cyanamide when it has been bombarded by uranium fission fragments. The damage of inert materials such as mica[9,10] and uranium oxide[11,12] has been described by others.

An experimental method developed by L. T. Chadderton of this Laboratory was used: silver cyanamide crystals in contact with thin foils of natural uranium were irradiated in the Harwell *Bepo* reactor for periods of 1 min.–10 hr. at a pile factor of 12. The grids were then examined by transmission electron microscopy as described above.

Fig. 2C shows a typical result of the irradiation. The light dashes and dots show that where each fragment has penetrated the crystal, material has evaporated or diffused away. The longest tracks represent paths of fragments travelling almost in the plane of the crystal, while dots indicate fragments that penetrated the crystal vertically. The mean track diameter is about 120 Å. The silver originally present in the volume occupied by a track has invariably diffused away from the track. On a few micrographs, an occasional fragment is seen to have caused greater damage than usual.

The greater part of the heat liberated by the passage of a fission fragment through a solid is due to ionization. Taking rough values of range and initial energy of a fragment in silver cyanamide to be 30μ and 80 MeV., the energy released is about 300 eV./Å. along a track. With the observed track radius of 60 Å., this gives a figure of about 650° C. (reached in about 5×10^{-11} sec.) for the maximum temperature of a molecule at the wall of a track. This implies that this is the maximum instantaneous temperature that a molecule can reach without decomposing: but the figure of 650° C. is necessarily very crude, because of the assumptions made in the calculation. However, the width of each light area is greater than in the inert materials, indicating that the damage is greater when additional heat is liberated by the decomposition of the material. This heat is of the same order as the heat supplied by the passage of the fragment, per unit length of track. It is not enough to produce explosion; this is consistent with estimates of limiting hot-spot sizes (10^3–10^5 Å.) for a number of explosives (see ref. 13 for further discussion).

The silver produced by the decomposition, in diffusing away from the sites where it is liberated, shows the same high mobility of the silver released from the crystals heated in the furnace.

Conclusion

The thermal decomposition studies have shown the importance of direct observation in investigating the physical processes involved. Nucleation at the interface between parent crystal and solid decomposition product has been observed only in the special case involving the initiation of holes of crystallographic shape. In general, the decomposition has been shown

to take place everywhere on the crystal surface; and there is a suggestion that surface steps have some influence on the decomposition. The silver produced by the decomposition is very mobile on the crystal surface. The study of fission fragment tracks shows that this mobility applies also to silver liberated within the body of the crystal. It also confirms the earlier conclusion that the 'hot-spots' or 'hot cylinders' produced by fission fragments are too small to initiate explosion.

One of us (H. M. M.-P.) would like to thank the Master and Fellows of Trinity College for the award of a research scholarship, and the Department of Scientific and Industrial Research for a maintenance grant.

[1] Bowden, F. P., and Yoffe, A. D., *Fast Reactions in Solids* (Butterworths, 1958). See also *Initiation and Growth of Explosion in Liquids and Solids* (Cambridge University Press, 1952).
[2] Bowden, F. P., and McAuslan, J., *Nature*, **178**, 408 (1956).
[3] Bowden, F. P., and Camp, M., *Proc. Eighth Intern. Symp. on Combustion*, California, 1960 (to be published).
[4] Sawkill, J., *Proc. Roy. Soc.*, A, **229**, 135 (1955).
[5] Camp, M., *Proc. Fourth Intern. Cong. for Electron Microscopy*, 1958 (Springer-Verlag, Berlin, 1960).
[6] Goodman, J. F., *Proc. Roy. Soc.*, A, **247**, 346 (1958).
[7] Forty, A. J., *Phil. Mag.*, **5**, 787 (1960); **6**, 587 (1961).
[8] Brotherton, T. D., and Lynn, J. W., *Chem. Revs.*, 841 (Oct. 1959).
[9] Silk, E. C. H., and Barnes, R. S., *Phil. Mag.*, **4**, 970 (1959).
[10] Bonfiglioni, G., Ferro, A., and Mojoni, A., U.S. Air Force A.R.D.C. Contract No. *AF*.61 (514)-1333 (1960).
[11] Noggle, T. S., and Stiegler, J. O., *J. App. Phys.*, **31**, 2199 (1960).
[12] Bierlein, T. K., and Mastel, B., *J. App. Phys.*, **31**, 2314 (1960).
[13] Bowden, F. P., and Singh, K., *Proc. Roy. Soc.*, A, **227**, 22 (1954).

TERNARY COMPOUND FORMATION IN THE SYSTEM $CaO - Al_2O_3 - MgO$

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THE quaternary system $CaO–Al_2O_3–SiO_2–MgO$ has been studied extensively at high temperatures because phase equilibria in this system provide basic knowledge of importance in understanding geological and industrial processes. Of the four ternary planes bounding the quaternary volume, the $CaO–Al_2O_3–MgO$ system is unique since its original and only investigators to date[1] found no evidence of ternary compound formation. Possibly, because of the apparently simple nature of the system and also its lack of geological significance, its long-established phase diagram has never been questioned. The system is, however, of considerable importance in understanding the potential compound formation in high-alumina cement, and work in this connexion has led to the discovery of two ternary compounds having the approximate compositions $7CaO.5Al_2O_3.MgO$ and $25CaO.17Al_2O_3.8MgO$.

The discovery of these compounds arose from the reported existence[2] of a quaternary compound, the composition of which was believed to be $6CaO.4Al_2O_3.MgO.SiO_2$. Attempts to confirm this reported formula were unsuccessful and led to an extensive investigation of adjoining compositions. In the course of this work, high-temperature microscope observations revealed that a homogeneous compound of composition $7CaO.5Al_2O_3.MgO$ could be prepared having optical and X-ray properties similar to those of the alleged quaternary compound. Since then, further work has disclosed that this ternary compound may be metastable at all temperatures, and is certainly so at temperatures above the solidus of the ternary system (1,344·5° C.) since it is observed to melt at 1,332° C. Its significance, however, resides in its solid-solution behaviour in the presence of silica. Preliminary observations suggest that a discontinuous series of solid solutions exist between $7CaO.5Al_2O_3.MgO$ and the compound gehlenite, $2CaO.Al_2O_3.SiO_2$. With increasing gehlenite content, these solid solutions first melt incongruently to yield a melt of $CaO.Al_2O_3$ and liquid, but congruent melting is established for compositions exceeding 40 per cent by weight gehlenite. It seems, therefore, that the quaternary compound reported might be accounted for as one of a series of solid solutions between two ternary compounds.

Following the initial discovery of the $7CaO.5Al_2O_3.MgO$ compound, a search was made in the system $CaO–Al_2O_3–MgO$ for a possible new primary phase field. Such a field was in fact located approximately by high-temperature microscopy and this was followed by a very precise quenching study of the indicated region. From a consideration of the course of crystallization of several of the compositions studied it became apparent that the very small primary phase field, which had been demarcated, could not be related to the $7CaO.5Al_2O_3.MgO$ compound.

It followed that a second ternary compound must exist in the system which bore a remarkable physical resemblance to the first. The two compounds in fact possess optical properties which are so similar that they cannot readily be distinguished by microscopic examination, although fortunately their X-ray diffraction patterns are quite distinct. The composition $3CaO.2Al_2O_3.MgO$ was at first assigned to the second of the two ternary compounds, but further examination of crystallization paths and homogeneity indicates that the almost contiguous and more complex composition $25CaO.17Al_2O_3.8MgO$ is more certainly correct. The similarity of optical properties suggested that there might be some solid-solution relationship between the two ternary compounds, but their separate co-existence has been checked by X-ray examination. As both compounds decompose on melting, their preparation in pure form presents considerable difficulty.

The 25 : 17 : 8 compound can be prepared in bulk by complete fusion at 1,650° C., followed by annealing of the rapidly chilled liquid at 1,200° C. Even with this treatment, several attempts may be necessary to suppress the formation of some magnesium oxide which, as the primary phase for this composition, tends to precipitate rather readily from the liquid. All similar attempts at bulk preparation of the 7: 5: 1 compound have failed; such heat-treatments

Table 1. Phase Data

$7CaO.5Al_2O_3.MgO$: Melts at 1,332° C.; may be metastable below this temperature with respect to $CaO.Al_2O_3$, $12CaO.7Al_2O_3$, and MgO.

$25CaO.17Al_2O_3.8MgO$: Melts incongruently at 1,350·5° C. with formation of $CaO.Al_2O_3$, MgO and liquid. Primary phase field terminates at:

(1) Reaction point; temperature 1,350·5° C. Equilibrium phases: $25CaO.17Al_2O_3.8MgO$, $CaO.Al_2O_3$, MgO and liquid. Composition (weight per cent): 42·6 CaO, 51·3 Al_2O_3, 6·1 MgO.

(2) Eutectic; temperature 1,346° C. Equilibrium phases: $25CaO.17Al_2O_3.8MgO$, $12CaO.7Al_2O_3$, MgO and liquid. Composition (weight per cent): 44·4 CaO, 49·9 Al_2O_3, 5·7 MgO.

(3) Eutectic; temperature 1,344·5° C. Equilibrium phases: $25CaO.17Al_2O_3.8MgO$, $12CaO.7Al_2O_3$, $CaO.Al_2O_3$ and liquid. Composition (weight per cent): 43·8 CaO, 50·9 Al_2O_3, 5·3 MgO.

Table 2. Optical Data

Composition	Refractive index (sodium light)	Birefringence	Optical character	Habit
$7CaO.5Al_2O_3.MgO$	$\alpha = 1{\cdot}671$ $\gamma = 1{\cdot}674$	Weak anomalous interference	Biaxial negative	Lamellar
$25CaO.17Al_2O_3.8MgO$	$\alpha = 1{\cdot}675$ $\gamma = 1{\cdot}678$			

have yielded a mixture of the 25 : 17 : 8 compound, $CaO.Al_2O_3$, and either $12CaO.7Al_2O_3$ or 'unstable $5CaO.3Al_2O_3$'. The latter compound has at various times been confused with the alleged quaternary compound $6CaO.4Al_2O_3.MgO.SiO_2$, but its separate existence has previously been established[3].

The 7 : 5 : 1 compound has only been isolated in minute quantity by carefully controlled supercooling of the melt conducted under continuous observation with the high-temperature microscope. Even by this technique it is only with the greatest difficulty that it has been found possible to grow single crystals of the compound, which exhibits a marked lamellar habit. The majority of compositions being studied at present in the hypothetical solid-solution series $7CaO.5Al_2O_3.MgO - 2CaO.Al_2O_3.SiO_2$ are,

Table 3. X-ray Powder Data

Because of limitations on space, only the principal X-ray spacings for the two compounds are given here. Full powder-diffraction data are available on private application. The *d*-values here as *VS* (very strong), *S* (strong), *M* (medium) were obtained from measurements in a Guinier-type focusing camera, 114 mm. diameter, with copper $K\alpha$ radiation

d (Å.)	*I*	*d*	*I*	*d*	*I*	*d*	*I*
			$7CaO.5Al_2O_3.MgO$				
3·72	*S*	2·776	*M*	2·145	*M*	1·802	*M*
3·36	*M*	2·724	*M*	2·086	*M*	1·784	*M*
3·09	*S*	2·473	*S*	2·032	*M*	1·764	*M*
2·93	*S*	2·390	*M*	1·966	*M*	1·524	*M*
2·89	*VS*	2·337	*S*	1·935	*M*	1·492	*M*
			$25CaO.17Al_2O_3.8MgO$				
4·21	*M*	2·682	*M*	2·029	*M*	1·681	*M*
4·07	*M*	2·602	*M*	2·008	*S*	1·669	*M*
3·91	*S*	2·493	*S*	1·955	*M*	1·646	*M*
3·79	*M*	2·478	*S*	1·942	*M*	1·629	*M*
3·72	*S*	2·460	*S*	1·937	*M*	1·626	*M*
3·63	*M*	2·404	*S*	1·927	*M*	1·619	*M*
3·58	*M*	2·378	*M*	1·913	*M*	1·574	*M*
3·10	*VS*	2·342	*M*	1·867	*M*	1·523	*VS*
2·89	*VS*	2·299	*M*	1·858	*M*	1·494	*M*
2·814	*M*	2·288	*M*	1·814	*M*	1·482	*M*
2·800	*M*	2·260	*M*	1·789	*VS*	1·464	*S*
2·784	*S*	2·211	*M*	1·765	*S*	1·454	*M*
2·772	*S*	2·153	*M*	1·734	*M*	1·442	*M*
2·723	*VS*	2·147	*M*	1·720	*M*	1·422	*S*
2·708	*M*	2·075	*M*	1·705	*S*		

however, susceptible to bulk preparation by the method described.

Although I hope to publish a more complete description of this work eventually, it seems desirable to present a summary of the available information which is given in Tables 1, 2 and 3.

This information may be particularly useful if it is found that analogous ternary compounds exist in which FeO substitutes for MgO and Fe_2O_3 for Al_2O_3, as seems not unlikely.

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[1] Rankin, G. A., and Merwin, H. E., *J. Amer. Chem. Soc.*, **38**, 568 (1916).
[2] Parker, T. W., Proc. Third Internat. Symp. on the Chemistry of Cement, London (1952), 487 (Cement and Concrete Association, London, 1954).
[3] Aruja, E., *Acta Cryst.*, **10**, 337 (1957).

FORMATION OF CHALCOPYRITE BY REACTION BETWEEN CHALCOCITE AND PYRRHOTITE IN COLD SOLUTION

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THE simple metallic sulphides are readily precipitated from solution by the sulphide ion. These sulphides have also been precipitated from solutions by the action of sulphate-reducing bacteria[1].

Up to the present, however, the complex sulphides have been synthesized only at higher temperatures either by the reaction of elemental sulphur, copper and iron, or by solutions containing one or more of the constituents acting on the metals or their sulphides. Roy[2] showed that bornite situated along grain boundaries in pyrite changes to chalcopyrite when heated at 100–175° C. for 3–5 days. Park[3] carried out experiments on copper compounds—his work on sulphides was concerned with passing steam, steam and carbon dioxide, and steam and hydrogen sulphide over chalcopyrite and chalcocite. Schouten's[4] work involved the action of concentrated aqueous copper solutions on various sulphides, and showed that chalcopyrite can form in a few hours on marcasite, pyrite, and pyrrhotite at atmospheric pressure and at about 100° C.

Gill[5] quotes results using solid blocks of simple sulphides of iron and copper at temperatures ranging from 350° to 650° C. for periods of 3–48 hr. Bornite and chalcopyrite were formed.

The present attempts at the formation of the complex sulphide differ from the previous work in that they were carried out at room temperature, and that either distilled water or sea-water played a part in the reaction.

It was decided to attempt the formation of the complex sulphide in this manner when, during the

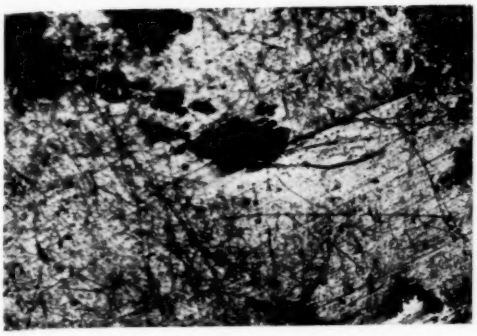

Fig. 1. Continuous coating of chalcopyrite on polished surface of chalcocite block (× 40)

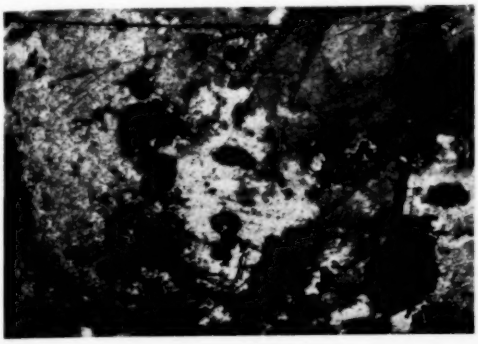

Fig. 2. Patchy coating of chalcopyrite on polished surface of chalcocite block. Chalcopyrite, dark grey; chalcocite, light grey (× 40)

course of preparation of blocks of sulphides for high-temperature synthesis, it was noted that, after about 5 min. contact between the wet ground faces of pyrrhotite (FeS) and chalcocite (Cu_2S), sufficient iron had been transferred to the chalcocite to give it a bronze colour on the ground surface. It was supposed that this iron had been removed from the surface of the FeS at an accelerated rate due to the presence of the water film at the interface, and that there was a possibility of a reaction with Cu_2S to form one of the copper iron sulphides.

Blocks about 1 mm. across were cut from naturally occurring chalcocite (Cu_2S), and pyrite (FeS_2), as well as from artificial pyrrhotite (FeS) made from iron filings and sulphur. A face was polished on these, and they were examined under an ore microscope to ensure that no other sulphides were present. Pairs of these minerals giving the combinations pyrrhotite–chalcocite and pyrite–chalcocite were held firmly together with their polished faces in contact, using clips made from heavy-gauge stainless steel wire.

A pyrrhotite–chalcocite and a pyrite–chalcocite pair were placed in a beaker of distilled water, and the same pairs placed in a beaker of artificial sea-water. Both beakers were allowed to stand at room temperature for five days. During this time it was observed that the pyrrhotite in both beakers formed a rim of hydrated iron oxide at the interface of the blocks within a few hours. After the first day a deposit of hydrated oxide was spread evenly over the bottom of both beakers. No apparent alteration took place on the exposed surface of the chalcocite blocks.

After five days the blocks were removed from their solutions and separated. The pyrite–chalcocite pairs in both sea-water and distilled water showed no reaction along the polished interface. The pyrrhotite–chalcocite pairs both had a brass-yellow deposit on the surface of the chalcocite. This deposit had covered the whole polished surface of the chalcocite from the distilled water (Fig. 1) but was patchy on the chalcocite from sea-water (Fig. 2).

In neither case was it found necessary to re-polish the chalcocite blocks for examination under the microscope; the new mineral had formed on the chalcocite, preserving the surface polish and scratches perfectly (Figs. 1 and 2). However, the surfaces of the pyrrhotite blocks had become etched, and had lost the original texture shown in the polished section (Figs. 3 and 4); but no new mineral had formed on their surfaces. The newly formed mineral was identified by X-ray diffraction as chalcopyrite ($CuFeS_2$). This mineral had not formed an intermediate layer between the two blocks—it had completely replaced part of the chalcocite; some of it had extended over the edges of the polished surfaces, along the sides of the chalcocite blocks. No other parts of the blocks exposed to the solutions showed any formation of chalcopyrite or any other change from the original material.

The depth of penetration of the chalcopyrite into the blocks of chalcocite was measured by cutting and polishing them in a plane normal to the polished surfaces. Microscopic examination at 1,100 magnifica-

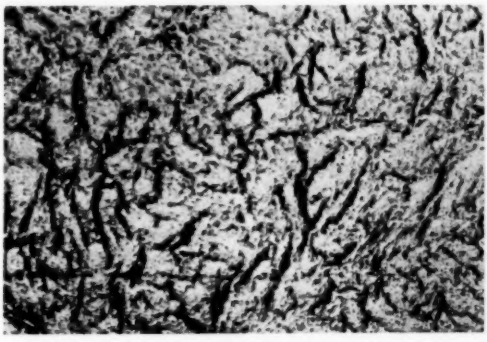

Fig. 3. Polished surface of pyrrhotite block before reaction (× 40)

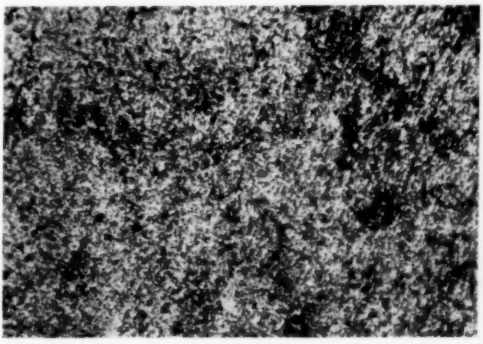

Fig. 4. Etched surface of pyrrhotite block after reaction (× 40)

tions showed that the penetration was highly irregular. It ranged from a sub-microscopic thickness to a depth of 0·11 mm. in the specimen placed in sea-water and to a depth of 0·13 mm. in that placed in distilled water. In one place chalcopyrite had formed to a depth of at least 1·5 mm. along a microscopic fracture.

It is of interest that, in these experiments, the iron moved into the copper sulphide, whereas Gill[5] found that the copper moved into the iron at a greater rate than the iron moved into the copper.

A block of chalcocite held in contact with a block of pyrrhotite for one month in an evacuated tube at room temperature showed a very faint brass-yellow tarnish on a small area of its surface, indicating that slight reaction had taken place even without the presence of a film of water between the blocks.

Some blocks of chalcocite were immersed in a cold solution containing ferrous ions, and another containing both ferrous and cupric ions, for ten days. No reaction was observed in any of the blocks. This seems to indicate that the reaction is not a result of general solution of iron from pyrrhotite and its subsequent reaction with chalcocite.

An electrolyte is evidently necessary for the reactions to proceed at a reasonable speed at room temperature. Both Cu_2S and FeS have very low solubilities:

Cu_2S, at 16° – 18° C.	2×10^{-47}
FeS, at 18° C.	$3{\cdot}7 \times 10^{-19}$

so that solution and recombination in the usual sense do not offer a reasonable explanation for the reaction because the quantity of $CuFeS_2$ is greater than could be expected to form from dissolved Cu_2S and FeS in the period involved in the experiment. Furthermore, a reaction was observed between Cu_2S and FeS even in the dry state. When the two sulphide blocks are brought into contact in water an electrolytic cell is formed, the e.m.f. of which, measured on a Cambridge *p*H meter, was found to be 0·51 V. This is responsible for the accelerated removal of Fe^{++} ions from the FeS and their transfer to the surface of the Cu_2S block to form the complex sulphide.

Both FeS and Cu_2S are capable of a high degree of non-stoichiometry. If non-stoichiometry is to persist in a crystal, electrical neutrality must be maintained —cation vacancies must be accompanied by a deficit of electrons to maintain neutrality—and therefore the degree of the non-stoichiometry is directly related to the diffusion properties as well as to the electronic properties of the crystals.

The behaviour of FeS in particular has been extensively studied, and it is known to be able to stand extreme departures from stoichiometry in the form of cation vacancies, without a change of phase. It would therefore be expected that where such great departures from stoichiometry are present they would be accompanied by a high diffusion constant.

Stone[6] has suggested a structure-sensitive diffusion by way of grain boundaries or Smekal cracks due to different mosaic structures in crystals. Barrer[7] gives some of the causes of this type of cracking in crystals along the surfaces of which diffusion can take place. As surface diffusion is much faster than volume diffusion it is possible that less activation energy is required for diffusion via cracks than via lattice vacancies.

Stone also suggests that the presence of impurities having different valencies from those of the normal crystal ions can have a marked effect in lowering the activation energy. If a divalent cation is substituted for a monovalent one in a crystal, electrical neutrality is maintained only by the formation of a cation vacancy. Cation vacancies of this type are present at all temperatures, and at the same concentration, and consequently their effect on diffusion will be much greater at lower temperatures at which there are fewer high-temperature vacancies.

The FeS used in this work is made up of randomly oriented small crystals the average size of which is 0·3 mm. The material has an irregular texture on the polished surface (Fig. 4), apparently the result of fractures developed during the cooling of an aggregate of anisotropic crystals. A semi-quantitative X-ray spectrographic analysis shows the impurities are: zinc, 0·5–1 per cent; copper, 0·1–0·5 per cent; lead, 0·1–0·3 per cent; arsenic, 0·05–0·1 per cent; manganese, 0·05–0·1 per cent; tin, trace; nickel, trace.

The Cu_2S used contains traces of nickel and iron as well as about 0·1–0·3 per cent bismuth. It is a polycrystalline aggregate having an average grain-size of about 0·6 mm. No cracks were observed on the polished surface at a magnification of 600, but the nature of the penetration of $CuFeS_2$ indicates that some diffusion has taken place along submicroscopic cracks as well as along grain boundaries.

The suggestions advanced by Stone suggest mechanisms by which low-temperature diffusion may take place. Both his conditions are fulfilled; these are the presence of impurities having a different valency and a large number of grain boundaries and fractures to act as channels for diffusion. In addition, the high degree of non-stoichiometry in these compounds enhances the possibility of diffusion at low temperature.

Once the iron has been removed from the FeS and brought to the surface of the Cu_2S, the complex sulphide will form because its lattice energy is greater than that of Cu_2S; the reaction goes in the direction where the free-energy change is negative.

Calculations of the unoccupied volume of the Cu_2S structure show that it has about 24 per cent unoccupied space compared with about 19 per cent for $CuFeS_2$. If the unoccupied volume bears an inverse relationship to the lattice energy, then it would be expected that $CuFeS_2$ would form at the expense of Cu_2S.

If this theory is valid, $CuFeS_2$ would form in preference to Cu_5FeS_4 (bornite), which has an unoccupied volume of about 23 per cent. Similarly its formation would be expected before the other copper–iron sulphides, cubanite ($CuFe_2S_3$) or valleriite ($Cu_3Fe_4S_5$), the unoccupied volumes of which are 22 and 41 per cent respectively.

I wish to thank Mr. L. L. Hawes of the Department of Chemistry, School of General Studies, Australian National University, for his helpful advice on some aspects of this work, and to acknowledge the help of G. J. G. Greaves, of the Bureau of Mineral Resources, in the study of the literature on sulphide synthesis. The photographs were taken by J. Zawartko, of the Bureau of Mineral Resources.

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[1] Baas-Becking, L. G. M., *Econ. Geol.* (in the press).
[2] Roy, J. E., *Econ. Geol.*, **25**, 433 (1930).
[3] Park, C. F., *Econ. Geol.*, **26**, 857 (1931).
[4] Schouten, C., *Econ. Geol.*, **29**, 611 (1934).
[5] Gill, J. E., *Report Twenty-first Sess. Int. Geol. Cong., Copenhagen*, Part 16, 209 (1960).
[6] Stone, F. S., *Lattice Defects in Ionic Crystals—Chemistry of the Solid State*, 20 (Butterworth, 1955).
[7] Barrer, R. M., *Diffusion in and through Solids*, 313 (Cambridge, 1951).

CRYSTALLIZATION KINETICS IN POLYVINYLCHLORIDE AND THERMOELASTIC RELAXATION INDUCED BY ULTRASONICS

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IT is still an open problem to determine with certainty the molecular characteristics governing the crystallization kinetics in long-chain polymers. The importance of different elements has been recognized, such as length of repeating unit in the chain, kind and dimension of lateral substituent groups, geometrical irregularity in which they can be placed along the chains, etc.[1]. As Kitaigorodski's work[2] on monomeric organic crystals suggests, the molecular packing efficiency also has to be considered. It may be negligible for molecules with a very awkward shape, and crystallization will not occur. The packing efficiency depends also on the stereochemical regularity of the molecules. On the other hand, it has been observed[1] that this efficiency can be lower in the regular arrangements for any shape of molecule, so that dearth of packing efficiency remains a not very convincing cause of failure to crystallize. Furthermore, there are polymers in which the chain carbon atom carries two lateral groups of different size, for example, polyvinyl alcohol $(-CH_2CHOH-)_n$, and these reach crystalline form easier than other polymers with the same deficiency in regularity of position, along the chain, of substituent atoms having almost equal size. Besides the approximate criterion requiring, in connexion with substituent group sizes, some geometrical regularity in their position in space, it is not possible to determine whether a long-chain polymer will crystallize at all, and if so, how readily.

It seems reasonable, therefore, that for polymers regarded as non-crystalline, the crystallization is not really impossible, but only the right conditions to crystallize are not found during the preparation of the material. I have used polyvinylchloride $(-CH_2-CHCl-)_n$ in the form of 500 per cent stretched fibre with high homogeneity characteristics (molecular weight approximately 60,000) in order to see whether the amount of crystalline material, normally very low (about 10 per cent before stretching, about 40 per cent after stretching in hot water and suddenly quenched), can still be increased in drawn material and in order to get information on the rate of this transformation.

In these fibres, assuming that crystallization is favoured especially by the parallel orientation of molecules induced by the drawing process, there is no reason to assume that chain folding is the normal case[3], and I shall not consider the presence of morphological structures like spherulites, the crystallinity comprising crystallites (or fringed micelles) embedded in the amorphous material and oriented with the carbon chains along the fibre axis. The fibre has been radiated with 1 Mc./s. ultrasonics for different time-intervals t and different temperatures T_B of the water-bath in which the samples were placed during the radiation. For an equal radiation time t, the greatest increase in crystallinity has been found at $T_B \simeq 90°$ C., that is, the temperature where attenuation measurements show a maximum in ultrasonic absorption by the polymer[4]. (I have not carried out systematic measurements above this temperature.)

In Fig. 1 the observed increase is plotted in percentage weight of crystalline material versus the radiation time t ($T_B = 90°$ C.) as measured by X-ray analysis. Detailed information has already been given[5,6] on these measurements and on appreciable effects on the mechanical and thermoelastic properties of fibre due to this increase in crystallinity[7].

I shall discuss here briefly what kind of useful information about the crystallization kinetics one can get from a knowledge of relaxation phenomena inside the fibre and from the growing modality of crystallite under ultrasonic radiation. Information and references concerning these phenomena in polycrystalline solid material are given in ref. 8. I should like to mention the importance of the thermoelastic relaxation at the frequency used here. At this frequency the molecular motions are taking place between the isothermal limit and the adiabatic limit, and flow of heat between adjacent crystallites arises from internal friction. The ultrasonic radiation induces strains which are macroscopically homogeneous ($\lambda_{us.} \simeq 0{\cdot}2$ cm.). The elastic anisotropy of single crystallites embedded in the amorphous material, and their irregular and discontinuous arrangement, present also in this case of strong orientation along the fibre axis, renders it almost impossible for these

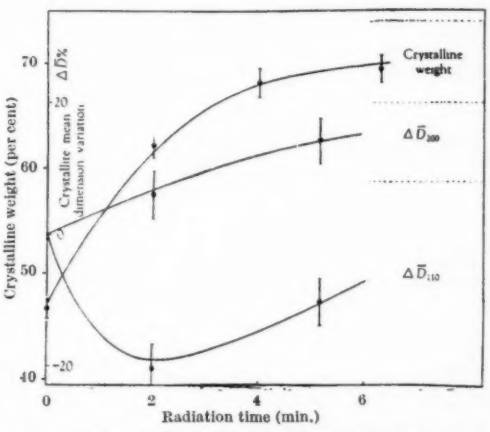

Fig. 1. Vertical axis left-hand scale gives crystalline weight per cent; the right-hand scale gives the change $\Delta\bar{D}$ per cent in mean crystallite dimensions $\bar{D}_{200}$ and $\bar{D}_{110}$, respectively, in the direction normal to the (200) and (110) planes, versus time of radiation. Dotted lines represent the limiting values for each quantity considered as obtained by fibre annealing and shrinkage at 150° C. in water

strains to be also microscopically homogeneous. Beside the flow of heat associated with these stress fluctuations, one has to consider also irreversible displacements in crystallite positions. These displacements can play a leading part in the crystallization process.

In order to account for the experimental observations on microscopic structure, one has to accept as true that inside the amorphous material many chain segments, all adjacent and already ordered parallel by stretching, have only a very low propensity to assume a packed configuration and consequently to crystallize. The reason for this, apart from molecular irregularity, is due I think to the fact that the movements of chain segments are hindered by the very low mobility of crystallites from which these segments ramify. The mutual distances between chain segments cannot be reduced by thermal oscillations, which do not affect the crystallite position sufficiently. From this description it seems clear why the 'effective' packing efficiency depends in a non-uniform way on regularity of chain structure, as the analysis of different polymers shows. In fact, some polymers may have, inside an amorphous region, almost parallel chain segments with quite regular structure and lateral groups of equal dimensions, but without the ability to move and, therefore, to form new nuclei, because of the very high friction associated with the smallest displacement of the binding crystallites. The chain segments will remain frozen in the positions assumed in the fibre production process, and crystallization will be arrested at low values for any interval of some limited time-scale of observation. In other polymers, also with different dimensions of lateral groups or some irregularity in their disposition along the chain, the friction for crystallite displacements may be very low, so that the relaxation processes bringing the chain segments to a close-packed configuration take place in a short time.

Accepting this picture, the ultrasonic action through change in local stress and temperature may be very relevant, as we have observed in polyvinylchloride. The rate of generation of new nuclei and their further growth strongly increase with the energy losses of the ultrasonic field. In polyvinylchloride the difference in substituent group dimensions is large (the Van der Waals's radii of hydrogen and chlorine atoms are respectively 1·10 Å. and 1·70 Å.). Atacticity, always present in a measure depending on the polymerization process used, may also prevent crystallization. On the other hand, the possibility of changing the degree of crystallinity to a very large extent, suggests that a more important element is the chain mobility and, therefore, the possibility for crystallites to realize irreversible displacements. This point of view is quite well supported by the experimental observation that, allowing the stretched polyvinylchloride fibres to recover their initial length in a thermal bath above the glassy transition temperature T_g, the amount of crystallinity may still increase up to 80 per cent in weight[6].

In Fig. 2 the general behaviour of the probability of nucleus generation, $P = \text{const.} \exp(-\Delta G/KT)$, is plotted versus temperature between the melting point T_m and the II-order temperature T_g. The width of the interval T_gS depends on chain mobility, strongly affecting the Gibbs free-energy variations ΔG. For chain segments inside the amorphous region, bound to not appreciably mobile crystallites, their

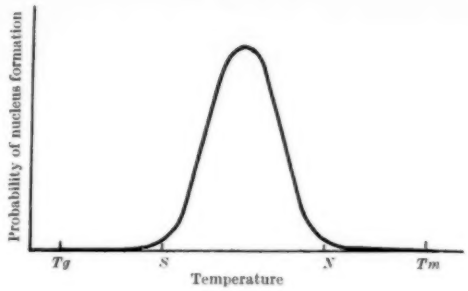

Fig. 2

mobility is very much reduced and the same applies to the probability of nucleus formation.

Such a strong influence of very low crystallite mobility on that of the amorphous chain segments, which constitutes the essential point in the microscopic model assumed here, may also operate above T_g, where the polymer is in a rubber-like state, provided that the fibre has bounded ends, as has been produced during ultrasonic radiation. In such a way fibre retraction is prevented; low mobility and strong orientation of chain segments in amorphous and crystalline regions are conserved. The thermoelastic relaxation induced by ultrasonics explains quite well the increase of crystallization with radiation time. If it is true that this relaxation governs the increase of crystallization with time, it becomes clear enough how the molecular characteristics can influence the crystallization process. They are essentially contributing to the same extent that the crystallite mobility and, consequently, that of the chains, is affected.

One has to observe that in the picture now presented, it is misleading to accept for the chain segments of the amorphous region, the possibility that all configurations consistent with the chain structure itself is approached. As a consequence, one has to change appreciably the evaluation of the configuration factor used in the Flory–Huggins theory and in the one-phase model of Frith and Tuckett[9], in order to determine the thermodynamic potentials.

In a general case, the generation of new nuclei may be described as taking place by two steps: in the first a parallel arrangement of chain segments is reached with high changes in entropy ΔS; in the second, the chains pack through small displacements, a monomer unit length at most, along their axis, in a configuration having a minimum internal energy U.

For stretched fibres, the parallel configuration is approached everywhere to a large extent, and the entropy contribution to the free-energy changes $\Delta F = \Delta U - T\Delta S$, originated by crystallization, is not appreciable ($\Delta S \simeq 0$). The increase in pressure and the relaxation phenomena induced by ultrasonics allow the energy barriers separating the amorphous from the crystalline configuration to be overcome. The possibility of new nuclei generated in already partially crystalline fibres must depend also on the extension of the amorphous regions, all interconnected, but approximately delimited by surrounding crystallites as set up in the stretching process. If the number of parallel chain segments in some of these regions is too low, no transformation of

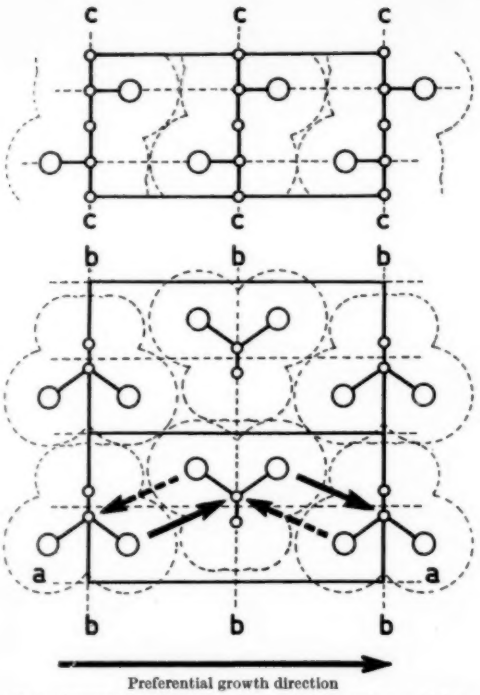

Fig. 3. Assumed crystalline structure with the axis $a = 10{\cdot}6 \pm 0{\cdot}1$, $b = 5{\cdot}4 \pm 0{\cdot}1$, $c = 5{\cdot}1 \pm 0{\cdot}1$ Å. A pair of strongly interacting dipoles is connected with → (in a different (00k) plane with respect to the other pair connected with – – –→)

embrions in new nuclei is possible and the crystallization will advance only by further growth of the confining crystallites. If the segment number is high enough, the variation in the Gibbs thermodynamic potential of n chain-segments changing to crystalline form, $\Delta G = An^{2/3} + Bn$, may have the surface contribution $An^{2/3}$ smaller than Bn. In this case, a nucleus may be formed, and it also will grow after ultrasonic radiation stops and with quenching below T_g, when the relaxation in the neighbouring binding crystallites is extremely low.

Interesting information on this point has been obtained[4] by measurements based on X-ray analysis. I assume that polyvinyl chloride crystallizes in the orthorhombic system *Pacm* as has been shown by Natta and Corradini[10], according to the scheme shown in Fig. 3.

From the integral line-breadth of the observed reflexions, the mean width of the crystallite $\overline{D}_{200}$, in the direction normal to the (200) plane, increases with radiation time t. On the contrary, the mean width $\overline{D}_{110}$ decreases with radiation even if some recovery may be observed for high values of the radiation time. These results, with the increase in crystalline material, indicated in Fig. 1, suggest the conclusion that, under ultrasonic action, the polymer crystallizes both with formation of new crystallites and growth of those already present. The evidence that crystallization takes place preferentially in the direction normal to the (200) plane (along the a-axis), as I have often observed, is very important, in order to understand what kind of intermolecular force is the most influential in this process. One may explain the existence of this preferred direction by considering the strong forces between carbon–chlorine dipoles of neighbouring chains. In fact, the preferential growth of crystallites along the a-axis offers the possibility of packing the greatest number of dipoles with electric moment orientation of smallest potential energy, the opposite poles of two neighbouring chains being at the shortest distances in the assumed crystalline structure.

Considering that $\overline{D}_{110}$ is always smaller than $\overline{D}_{200}$ (~ 50 Å. and ~ 60 Å. respectively) in the oriented or isotropic fibre states as obtained by stretching and shrinkage, this indicates clearly that in polyvinyl chloride crystallization the C—Cl dipole–dipole interaction is prevailing over the other Van der Waals's forces.

I thank Dr. M. Mammi, of Centro di Strutturistica Chimica del C.N.R., University of Padua, for essential help given in the analysis of the X-ray patterns carried out in that Institute and for many discussions. Thanks are due also to Dr. F. Smeraldi, technical director of Montecatini-Polymer, who has kindly furnished the polyvinyl chloride fibre prepared under known and reproducible conditions.

[1] Bunn, C. W., *J. App. Phys.*, **25**, 320 (1954).
[3] Kitaigorodski, A., *Acta Physicochimica, U.S.S.R.*, **22**, 309 (1947).
[3] Stuart, H. A., *Ann. New York Acad. Sci.*, **83**, 1 (1959).
[4] Maeda, Y., *J. Polymer Sci.*, **18**, 87 (1955).
[5] Nardi, V., and Pisent, G., *Proc. Third Intern. Cong. Acoustics* (Elsevier, Pub. Co., Amsterdam, 1959).
[6] Nardi, V., and Pisent, G. (in the press).
[7] Nardi, V., Italian Patent No. 20753 (2-12-1960).
[8] Kittel, C., *Reports on Progress in Physics*, **11**, 205 (1947).
[9] Stuart, H. A., *Die Physik der Hochpolymeren*, **3**, B, 543 (1955).
[10] Natta, G., and Corradini, P., *J. Polymer Sci.*, **20**, 251 (1956).

INTERACTION OF LIPIDS AND TRYPSIN IN DIMETHYL SULPHOXIDE–WATER SYSTEMS

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THE nature of the binding sites and binding forces between lipids and proteins is virtually unknown. The attempts that have been made to create model systems for the investigation of this binding have been hampered by solubility limitations. All the systems examined were heterogeneous and included: lipid colloidally dispersed in aqueous protein solution in the presence[1,2] or absence of dispersing agents[1,3], and lipid adsorbed on an inert solid surface and suspended in an aqueous protein solution[4]. The recent use of various non-aqueous lipid solvents in solvating proteins such as dimethyl sulphoxide

(DMS) and formamide[5], affords an opportunity of analysing lipid–protein complexing in a homogeneous system.

We have chosen trypsin for this work. It is particularly suitable because it has a relatively high solubility in water and in DMS and can be transferred from DMS to water with practically no loss in activity[5]. This transfer is necessary because the trypsin esterase activity can be determined only in aqueous medium. Preliminary efforts to demonstrate lipid–protein binding in DMS by observing changes in absorption spectra were unsuccessful. Other attempts to demonstrate strong lipid–protein interaction in DMS also failed. These included a continuous dialysis of lipid–protein DMS mixture against DMS and extraction of the mixture with petroleum ether. In each of these cases the lipid was removed easily. The only indication of an interaction between protein and lipid in DMS was the apparent decrease in enzyme activity.

Four different experiments were performed in observing the inhibition of enzyme activity by lipids:

(*a*) Enzyme and lipids were mixed simultaneously as follows: 3 mgm. of crystalline enzyme (Worthington) were dissolved overnight in 10 ml. of freshly distilled DMS. An 0·5 ml. aliquot of this solution was mixed with 0·5 ml. of a DMS solution of a lipid in concentrations varying from 0 to 4×10^{-3} *M* in DMS. The lipid–enzyme mixture was then added with stirring to 50 ml. of 0·02 *M tris* buffer at *p*H 7·90 which was 0·01 *M* in calcium chloride and 0·04 *M* in sodium chloride. Trypsin esterase activity was then determined by the usual procedure using benzoyl arginine ethyl ester as substrate.

The lipids used were stearic acid and a variety of carotenoids which were kindly supplied by Hoffman–La Roche, Nutley, New Jersey. The carotenoids were chosen to provide functional groups of differing polarity and because of their colour to make observations easier. Included were beta-apo-8′-carotenal *trans*-bixin, *cis-nor*bixin and vitamin *A*.

(*b*) A filtration experiment was performed as follows: Solutions were prepared and mixed as in (*a*). Before trypsin esterase activity was determined, however, the solution was filtered through a fine-pore (5μ) sintered-glass filter. All the colour was removed by this procedure.

(*c*) Mixing afterwards of lipid and enzyme: Solutions were prepared as in (*a*) except that the trypsin in DMS was added to the buffer solution followed by the addition of lipid in DMS.

(*d*) Later mixing of enzyme and colloidal carotenoid: Trypsin dissolved in DMS plus an equal volume of solvent were added to the buffer solution. This was followed by the addition of an aqueous colloidal solution of carotenoid which was prepared by introducing an acetone solution of the carotenoid into buffer and then evaporating the acetone *in vacuo*. The same final concentrations of carotenoid as in (*a*) and (*c*) were used.

The results are summarized in Fig. 1 and in Table 1. Among the carotenoids examined the greatest inhibition was observed with beta-apo-8′-carotenal. In Fig. 1, one can see an increase in inhibition with increasing lipid to protein molar ratio which follows a logarithmic course. This occurs only when the lipid and enzyme are premixed in the solvent and, therefore, added together to the buffer. On the other hand, on additions afterwards of lipid in the solvent or the addition of lipid in colloidal aqueous suspension to the buffer into which trypsin in the solvent had been

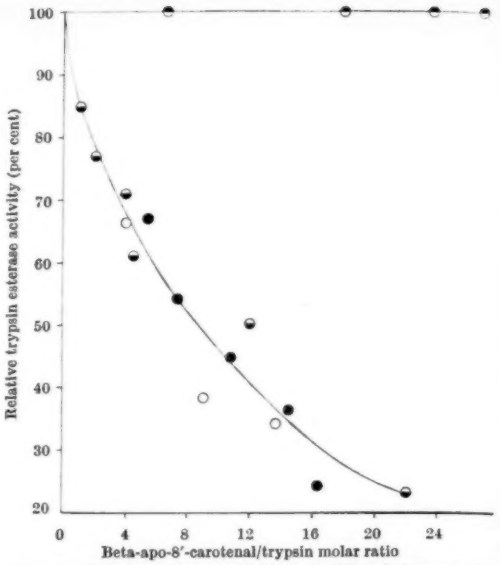

Fig. 1. Trypsin esterase activities at different beta-apo-8′-carotenal to trypsin molar ratios; ◓, subsequent addition of beta-apo-8′-carotenal in DMS to trypsin in DMS dispersed in buffer; ◒, simultaneous addition of beta-apo-8′-carotenal and trypsin in DMS to the buffer, first experiment; ○, same, second experiment; ●, simultaneous addition of beta-apo-8′-carotenal and trypsin in DMS to buffer and subsequent filtration

transferred before, there was no apparent reduction in activity. It is evident from the experiment in which the solutions were filtered after addition at the same time of lipid and enzyme to the buffer, that the filtrate showed approximately the same activity as that of the unfiltered material. Therefore, this indicates that the decrease in activity was the result of an aggregated lipid–protein complex of larger than colloidal size, making the enzyme unavailable for reaction. This was confirmed by experiments in which the precipitated complex was isolated in larger quantities. Only a small quantity (about 10 per cent) of the original activity was found in these complexes. At high lipid enzyme molar ratios, the activity determination became more erratic because of the fact that large amounts of enzyme were removed resulting in less accuracy. Other lipids inhibit trypsin esterase activity in a manner similar to that of beta-apo-8′-carotenal. In Table 1, the lipid to enzyme molar ratios required to reduce the enzyme activity to one-half of that in the absence of lipids are given. One can see a direct relationship between the extent of inhibition and the polarity of the lipid molecule[6].

Because of the relationship noted between the polarity of the lipid and extent of enzyme inhibition, activities were determined at various ionic strengths. Solutions of the buffer at *p*H 7·90 were prepared by varying the sodium chloride concentration to give values of ionic strength ranging from 0·065 to 0·50. Three different concentrations of beta-apo-8′-carotenal representing low, intermediate and high

Table 1. Molar Ratio of Lipid to Protein at 50 per cent Residual Esterase Activity

Lipid	Functional groups	Molar ratio
Beta-apo-8′carotenal	–CHO	8·3
Trans-bixin	–COOH, –$COOCH_3$	11·8
Stearic acid	–COOH	15·0
*Cis-nor*bixin	–COOH, –COOH	20·0
Vitamin *A*	–OH	66·7

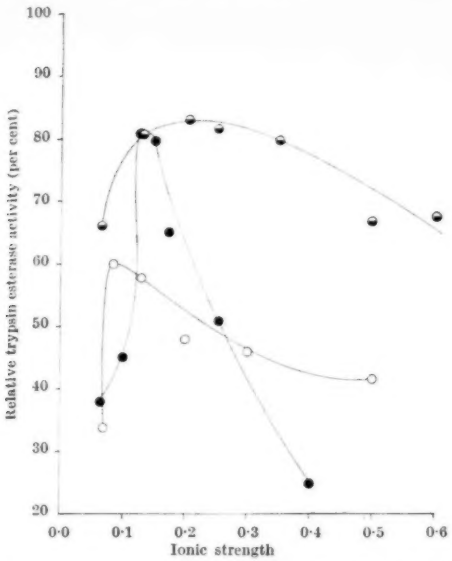

Fig. 2. Relative trypsin esterase activity at different ionic strengths and at different beta-apo-8′-carotenal to trypsin molar ratios; ◒, molar ratio 4 : 1; ●, molar ratio 9 : 1; ○, molar ratio 13·5 : 1

inhibition effects (beta-apo-8′-carotenal/trypsin molar ratio of 4 : 1, 9 : 1, 13·5 : 1) were examined in solutions of various ionic strengths. Trypsin esterase activity was determined as in 1(*a*).

The results are shown in Fig. 2. One can see two main features of the graphs: (*a*) the curves show maxima which appear at lower ionic strengths the higher the beta-apo-8′-carotenal/trypsin molar ratio; (*b*) the activities at an ionic strength of 0·065 followed the results of previous observations, that is, the activities decreased in a logarithmic fashion with the increasing beta-apo-8′-carotenal to trypsin molar ratio. A corollary to this is that the activity values at the maxima of the curves show the same trend.

This indicates that the interaction between protein and lipid is between the polar groups of the molecules or of the aggregate surfaces. At every molar ratio tried, a maximum appears in the ionic strengths of the medium versus activity curve. One may interpret this as two processes competing with each other. The first process which caused a rise in the relative activity with increasing ionic strength can be understood as the depression of interaction between the ionized surface of protein and the polar groups of lipid. One may interpret the second process, in which the activity decreased with increasing ionic strength, as the progressive aggregation of colloidal particles, that is, at high enough ionic strengths the depression of the charges on the protein surface not only decreased the interaction between the lipid and protein, but it also may have decreased the electrical double layer around the colloidal particle, thus enhancing aggregation.

The maxima in the activity curves, therefore, indicate the ionic strengths at which the second process starts to dominate the first. The fact that an inverse relation existed between the lipid to protein ratio and the ionic strength at which the maxima occurred supports this hypothesis.

It would appear that the decrease in enzyme activity was due chiefly to the aggregation of the lipid–protein complexes to larger than colloidal sizes.

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[1] Karrer, P., and Strauss, W., *Helv. Chim. Acta*, **21**, 1624 (1938).
[2] Sarda, L., Marchis-Mouren, G., and Desnuelle, P., *Biochem. Biophys. Acta*, **24**, 425 (1957).
[3] Traitskiland, G. V., and Tarasova, L., *Biokhimiya*, **20**, 19 (1955); *Chem. Abst.*, **49**, 9120 (1955).
[4] Avigan, J., *J. Biol. Chem.*, **234**, 787 (1959).
[5] Rees, E., and Singer, S., *Arch. Biochem. Biophys.*, **63**, 144 (1956); Vratsanos, S., *Arch. Biochem. Biophys.*, **90**, 132 (1960).
[6] Smyth, C. P., in Weissberger, A., *Physical Methods of Organic Chemistry*, **1**, Part 2, 1614 (Interscience Publ. Inc., New York, 1949).

RIGIDITY FACTOR OF GELATIN GELS

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PREVIOUS work[1–3] on gelatin fractions has disclosed an unidentified structural feature, termed the 'rigidity factor', which affects the rigidity of gelatin gels. Thus, it has been shown that variations between the rigidities of gels, set and matured either at 0° C. or 10° C., from different gelatin samples are related neither to differences in their molecular weights[2,3] (above a limiting viscosity number[4] $[\eta]$ in molar sodium chloride at *p*H 7 of about 40; that is, $\bar{M}_\mu \sim 115{,}000$ (ref. 5)) nor to possible differences in the average number of component chains per molecule[6]. Moreover, the considerable variation in gel rigidity (at high molecular weights) which can occur for gelatins derived by successive extraction from the same pretreated collagen is not accompanied by any apparent change in amino-acid composition[7]. The rigidity factor is reduced during thermal degradation in neutral or alkaline solutions[3]; but it is virtually unaffected by degradation in acid solution[3] or with enzymes or ultrasonics[8]. The present investigation was designed to discover whether the rigidity factor is related to the chain configuration, which would be reflected in the optical rotation of the gel.

Three gelatin samples of widely varying rigidity factor, but of the same high molecular weight and similar in chemical composition, were chosen for this investigation. Two of them (Nos. 76 and 127) were good-quality commercial lime-processed ox-hide gelatins; and the third (*AT*/1*a*), of very high rigidity, was prepared in the laboratory, as a neutral first extract from demineralized ossein (from the mixed bones of Indian cattle), by adaption of the accelerated alkaline pretreatment process of Ward[9]. The three gelatin samples would not differ significantly in amino-acid composition, since they were all derived from the same species of mammal[10]; and they would exhibit

the low isoionic point $pI \sim 5$ typical of gelatins prepared by an alkaline process[11], corresponding to the conversion of most of the amide groups of the glutamine and asparagine residues of collagen to carboxyl groups[12].

Optical rotation and rigidity measurements were carried out on gels, from these three samples, which had been prepared under identical conditions and carried through exactly the same schedule of changes in temperature. Each gelatin sol, containing 5·5 gm. protein/100 ml. at *p*H 7, was filtered through a No. 3 sintered-glass funnel and used to fill either a 1-dm. jacketed polarimeter tube or a type *A* rigidity tube[13] at 40° C., and then quenched rapidly to 0° C. and left to mature overnight. Readings were taken at 10-min. intervals over a period of 23 hr. 15 min.–24 hr. 45 min. at 0° C., so that the mean of the 10 readings corresponded to 24 hr. maturing at 0° C. The temperature was then raised as quickly as possible (during 30 min.) in 10 deg. C. stages, taking care never to exceed the required temperature. Readings were taken at each temperature, particularly (10-min. intervals) over the 1½-hr. period corresponding to a mean of 3 hr. at 10, 20 and 30° C., and to 65 min. at 40° C. It was found in all cases that, over these 1½-hr. periods of measurement from 0 to 30° C., the optical rotation, as well as the rigidity[8], was changing very slowly and (within experimental error) linearly with time, so that the means of these observations were adequate for comparative purposes. At 40° C. there was no detectable change in optical rotation over the period of measurement. All temperatures were controlled to within ± 0·1 deg. C.

The optical rotation measurements were made for the sodium *D* line using a Bellingham and Stanley model *A* visual polarimeter with a screened light source in a dark-room at a half-shade angle of 8°. The glass scales were illuminated weakly by 2·5-V. torch bulbs set behind ¼-in. green 'Perspex', so that the dark adaptation of the eye was not destroyed between successive measurements. Zero readings were obtained by washing and refilling the polarimeter tube with distilled water (via the thermometer side-arm), without disturbing the end-plates, and repeating the measurements at each temperature. Following the suggestion of Bates *et al.*[14], condensation on the end-plates at temperatures below the dew-point was avoided by the addition of 'Perspex' chambers, containing silica gel and closed by a second pair of glass windows, to the ends of the polarimeter tube. To facilitate rigidity measurements on the weaker gels, carbon tetrachloride coloured with a small amount of a solid dye, 'Waxoline Red *O.S.*', was used instead of mercury[13] as the capillary fluid, and a dual system of manometers, one containing mercury and the other carbon tetrachloride, was employed to obtain and to record the required wide range of applied pressures. Protein concentrations at 40° C. were determined in duplicate by evaporating 20-ml. portions of the excess gelatin sol to constant weight in an oven at 105° C.; the residues were then corrected for the weights of inorganic material present by moistening them with concentrated sulphuric acid, charring them over a low flame, and igniting them in an oven at 550° C. for 2 hr.

The optical rotation measurements are shown in Table 1 and Fig. 1. From the results for duplicate runs on gelatin 127, the coefficient of variation of the specific rotation $[\alpha]_D$ is less than 1 per cent from 0 to 30° C. and only about 0·15 per cent at 40° C. Therefore, the differences between the specific rotations of the three gelatins are significant at each temperature. Sample 76/2/1 was a 50 per cent top fraction of an 87 per cent middle fraction obtained from gelatin 76 by coacervation with ethanol[15]. The specific rotation results for 76/2/1 are not significantly different, at any temperature, from those of the original gelatin 76 indicating that, at least for material of relatively high average molecular weight, the differences in molecular weight distribution are unimportant.

Table 1. Specific Rotation of Gelatin Gels and Sols

Gelatin No	76	76/2/1	127		*AT*/1*a*
$[\eta]$ in *M* NaCl, *p*H 7, 40° C.	61	63	64		62
Conc. gm. protein/100 ml.	5·591	5·452	5·461	5·504	5·365
*p*H	6·85	7·88	6·94	6·89	6·98
$-[\alpha]_D$ at 0° C.	301·9	306·5	329·3	326·9	334·1
10° C.	269·9	269·1	302·8	304·2	315·3
20° C.	208·7	212·0	257·3	255·9	264·8
30° C.	150·1	148·6	163·0	161·0	174·7
40° C.	141·3	141·4	144·2	143·9	144·6

The corresponding rigidity measurements for the three gelatins are given in Table 2. A slight increase of the apparent rigidity modulus *G* (that is, shear stress/shear strain) with strain was observed for some of the gels, and so measurements for all the gels were made at the same small maximum strain (at the tube wall) of 0·15 (or interpolated graphically to this value). Thus, although the values of *G* recorded in Table 2 are not true rigidity moduli, they should be close enough to the constant theoretical elastic moduli for comparative purposes[13].

When the specific rotations in Table 1 were plotted against the apparent rigidity moduli in Table 2, for the same gels after exactly the same treatment, the points lay on a single smooth curve, showing that $[\alpha]_D$ and *G* are closely related. In Fig. 2, a linear correlation is seen to exist between $-[\alpha]_D$ and *G*!, where the values of *G* in Table 2 have been corrected

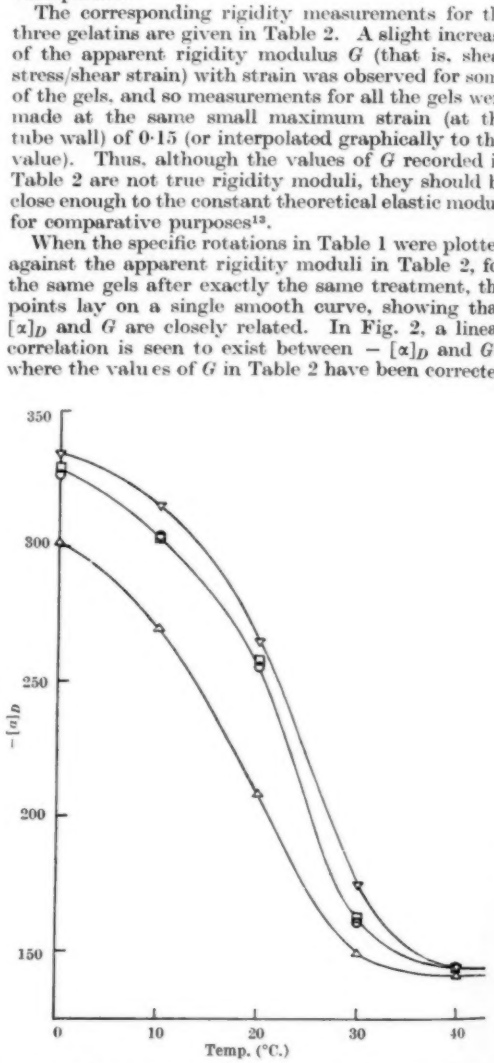

Fig. 1. Variation of specific rotation of three samples of gelatin: ▽, *AT*/1*a*; □, 127 run 1; ○, 127 run 2; △, 76

Table 2. RIGIDITY OF GELATIN GELS

Gelatin No.	76	127		*AT*/1*a*
Conc. gm. protein/100 ml.	5·416	5·499	5·488	5·160
*p*H	6·95	6·96	6·97	6·98
G dynes/cm.^{2} at 0° C.	71,070	108,380	108,580	119,960
10° C.	45,910	72,230	72,180	80,370
20° C.	12,360	36,310	35,040	43,340
30° C.	~ 6	~ 19	~ 14	188

to a concentration C of 5·50 gm. protein/100 ml. (taking $G \propto C^2$, over at least a small range of C, from the work of Ferry[16] and Ferry and Eldridge[17]).

The conditions of these experiments (that is, gelatins of similar chemical composition at the same concentration and *p*H) were chosen so that the contributions to the optical rotation from the individual amino-acid residues and from environmental factors were as nearly constant as possible at each temperature. The fairly close values of the specific rotation for sols of the three gelatins at 40° C. (Fig. 1), where the molecules are present as random coils[18], is good evidence that this constancy was virtually attained. Hence, the increase in magnitude of the specific rotation for the gels must be, essentially, a measure of the amount of regular structure or chain folding in the gel phase (providing that only one type of folding is present, and that there is no appreciable variation in environmental contributions as the temperature falls). Thus, it follows from the correlation in Fig. 2, that the rigidity modulus of a gelatin gel, for a given concentration, is also determined by the amount of chain folding, and the 'rigidity factor' defines the capacity of a gelatin molecule to fold in a regular manner.

There is substantial evidence, from X-ray diffraction work[19] and from the similarity of the melting temperatures for mixtures of collagen or gelatin with ethylene glycol[20], that this regular folding in gelatin gels is the same type of triple helix present in native collagen[21]. The unusual negative chain-configurational contributions to the specific rotation given both by gelatin gels[22,23] and by soluble collagens[23] are also consistent with this view. An isotropic gelatin gel must, therefore, contain lengths of randomly orientated triple helix (formed from portions of three gelatin molecules), which bind the molecules into a network and confer stiffness on the structure. There appears, at present, to be no theoretical model for this type of structure. Qualitatively, however, its mechanical behaviour would be expected to resemble that of a frozen unstrained rubber inasmuch as the modulus of the unorientated crystallites (or helices in the gel) would be superimposed on that of the amorphous network[24]. It is not yet known whether a higher rigidity factor corresponds to more, or to longer, triple helices.

Although it has been established that the rigidity factor of a gelatin gel measures the facility of the molecules to take part in reforming the collagen triple helix, the precise nature of the structural feature which is responsible remains obscure. Further evidence that the molecular structure of the three gelatin samples is not completely identical is shown by the small but significant differences between the specific rotations of the sols at 40° C. in the absence of chain order. It is possible that this small variation in the contributions to the specific rotation from the individual residues is due to subtle differences in amino-acid composition which are not detectable by the present experimental techniques. A more likely explanation, however, is that differences in the mutual interactions of the individual residues are produced by changes of amino-acid sequence along the gelatin chains: the rigidity factor would then be determined by the proportion of residues in sequences capable of forming the collagen fold.

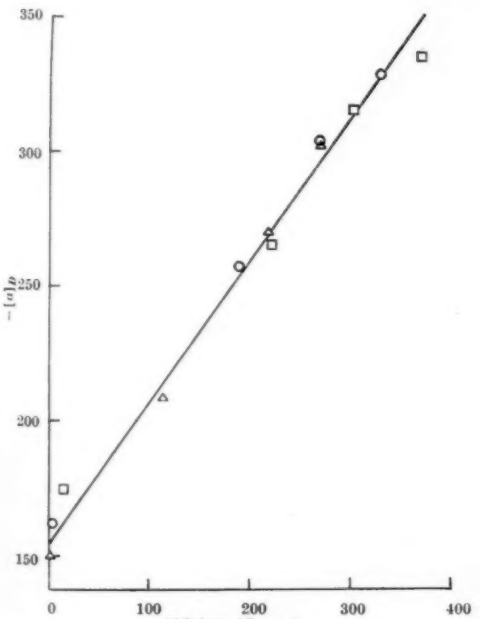

Fig. 2. Relation between specific rotation and rigidity for 5·5 per cent gelatin gels
△, 76; ○, 127; □ *AT*/1*a*

[1] Ward, A. G., *Nature*, **171**, 1099 (1953).
[2] Stainsby, G., Saunders, P. R., and Ward, A. G., *J. Polymer Sci.*, **12**, 325 (1954).
[3] Saunders, P. R., and Ward, A. G., *Nature*, **176**, 26 (1955).
[4] Stainsby, G., *Nature*, **169**, 662 (1952).
[5] Wootton, J. (to be published).
[6] Courts, A., *Biochem. J.*, **73**, 596 (1959).
[7] Eastoe, J. E., *Biochem. J.*, **61**, 589 (1955).
[8] Saunders, P. R., and Ward, A. G., *Recent Advances in Gelatin and Glue Research*, edit. Stainsby, G., 197 (Pergamon Press, London, 1957).
[9] Ward, A. G. (unpublished work).
[10] Eastoe, J. E., and Leach, A. A., *Recent Advances in Gelatin and Glue Research*, edit. Stainsby, G., 173 (Pergamon Press, London, 1957).
[11] Briefer, M., and Cohen, J. H., *Indust. Eng. Chem.*, **20**, 408 (1928). Briefer, M., *ibid.*, **21**, 266 (1929).
[12] Ames, W. M., *J. Soc. Chem. Indust.*, **63**, 277, 303 (1944). Bowes, J. H., *Research*, **4**, 155 (1951).
[13] Saunders, P. R., and Ward, A. G., *Proc. Second Int. Cong. Rheology*, 284 (Butterworths Scientific Publications, London, 1954).
[14] Bates, F. J., and associates, *Nat. Bur. Stand., Circular C*440, 103 (1942).
[15] Stainsby, G., *Faraday Soc. Discussion*, No. 18, 288 (1954).
[16] Ferry, J. D., *J. Amer. Chem. Soc.*, **70**, 2244 (1948).
[17] Ferry, J. D., and Eldridge, J. E., *J. Phys. Coll. Chem.*, **53**, 184 (1949).
[18] Boedtker, H., and Doty, P., *J. Phys. Chem.*, **58**, 968 (1954).
[19] Herrmann, K., Gerngross, O., and Abitz, W., *Z. physik. Chem.*, Abt. *B*, **10**, 371 (1930). Katz, J. R., Derksen, J. C., and Bon, W. F., *Rec. Trav. Chim. Pays-Bas*, **50**, 725 (1931).
[20] Flory, P. J., and Garrett, R. R., *J. Amer. Chem. Soc.*, **80**, 4836 (1958).
[21] Ramachandran, G. N., and Kartha, G., *Nature*, **176**, 593 (1955). See also review by Rich, A., and Crick, F. H. C., *Recent Advances in Gelatin and Glue Research*, edit. Stainsby, G. (Pergamon Press, London, 1957).
[22] Robinson, C., and Bott, M. J., *Nature*, **168**, 325 (1951). Robinson, C., *Nature and Structure of Collagen*, edit. Randall, J. T., and Jackson, S. F., 96 (Butterworths, London, 1953).
[23] Cohen, C., *J. Biophys. Biochem. Cytol.*, **1**, 203; *Nature*, **175**, 129 (1955).
[24] Leitner, M., *Trans. Farad. Soc.*, **51**, 1015 (1955). See also Treloar, L. R. G., *The Physics of Rubber Elasticity*, second ed. (Clarendon Press, Oxford, 1958).

TEICHOIC ACIDS AND THE STRUCTURE OF BACTERIAL WALLS

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THE chemical composition of the walls of a number of bacteria has been examined by many workers[1,2] and much interest has been expressed in the possible function of the various components. Electron microscopy of stained sections of whole cells of *Bacillus subtilis* has shown that the wall of this organism appears to be composed of three layers (for example, ref. 3), although the interpretation of such micrographs may be ambiguous. We have now examined stained sections of the isolated walls of *Lactobacillus arabinosus*, *L. casei*, *L. fermentii*, *B. subtilis* and *B. cereus*, and observed three layers in each case.

Vigorous extraction of lyophilized walls of *L. arabinosus* completely removes the teichoic acid and a polysaccharide containing rhamnose, galactose and glucosamine[4]. After such extraction, the insoluble residue (50 per cent of the original weight of wall) consisted of the mucopeptide component, which is composed of alanine, glutamic acid, diaminopimelic acid, glucosamine and muramic acid; all the glucose, rhamnose, galactose and teichoic acid which were originally present in the wall had been removed. This mucopeptide material contained about 0·4 per cent of phosphorus, which was not present as teichoic acid[4]. The nature of the phosphorus is unknown, but it is of interest that after complete removal of teichoic acid from the walls of *Staphylococcus aureus* a similar quantity of phosphorus remained[5]. Furthermore, some twelve species of Gram-positive bacteria containing no teichoic acid in their walls nevertheless contained small amounts of phosphorus (0·2–0·6 per cent) chemically bound in the wall.

Electron microscopical examination of shadowed specimens of the mucopeptide from *L. arabinosus* showed that it retained the shape of the original cell wall, indicating that in these walls the mucopeptide is the component responsible for the structural integrity of the wall, the teichoic acid and polysaccharide being of minor importance in this respect. Similar results were obtained on extracted walls from *B. subtilis* which contained only traces of teichoic acid. This finding is similar to that of Park and Hancock[6], who showed that the residual material obtained after exhaustive extraction and enzymic digestion of whole cells of *Staph. aureus* had the composition of a mucopeptide and the appearance of cell walls. Moreover, we have observed that stained sections of extracted walls of *L. arabinosus* possess a three-layer structure similar to that of the original walls. This suggests that the mucopeptide is not homogeneous, a finding which is in accord with views expressed by others[7,8] on the basis of chemical investigations. The appearance of walls before and after removal of teichoic acid is shown in Fig. 1.

In view of modern opinion that walls are not solely rigid supports for the cell, it is interesting to consider possible functions of wall teichoic acids. As they do not contribute significantly to the rigidity of the wall, it is possible that they are concerned in cell metabolism. After isolation, they have charged phospho-mono- and di-ester groups and also free amino-groups[9,10]. Thus it might be expected that the provision of charged groups is one of the functions of teichoic acids. Consequently, it was necessary to investigate the manner in which teichoic acid is bound to the cell wall, and to determine whether the charged groups of teichoic acid exist as such in the wall structure.

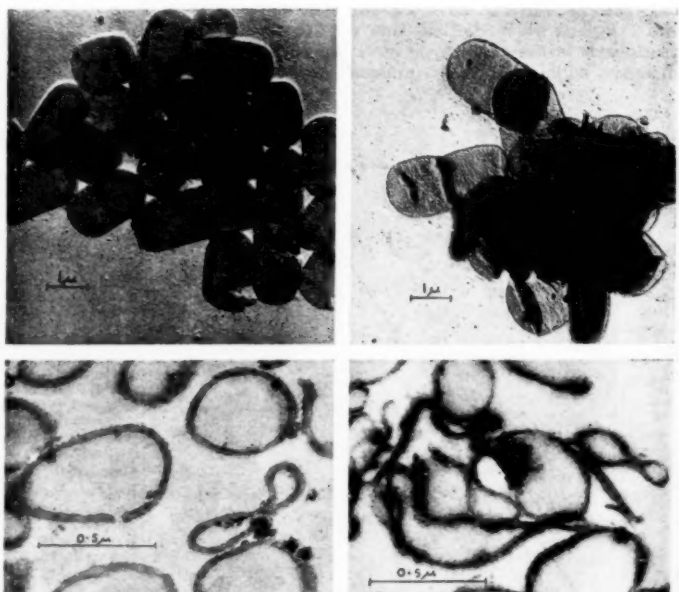

Fig. 1. Top left, walls of *L. arabinosus*; top right, walls of *L. arabinosus* after removal of teichoic acid and soluble polysaccharide; bottom left, section of walls of *L. arabinosus* stained with permanganate; bottom right, section of walls of *L. arabinosus* after removal of teichoic acid and soluble polysaccharide

It is unlikely that teichoic acids are bound to the walls by amide linkages involving the amino-groups of the D-alanine residues. These residues are joined to the teichoic acid molecule through exceptionally labile ester linkages which are nevertheless largely unaffected by the acid extraction procedures. It is improbable that amides would be hydrolysed under conditions which do not hydrolyse these ester linkages. Extraction of teichoic acid was effected more readily by trichloroacetic acid than by any of the other treatments described (Table 1). However, the amount of alanine released was greater in alkaline than in acidic or neutral solution, and the molar ratio of alanine liberated : teichoic acid phosphorus liberated was very much higher in alkaline than in acidic solution. For example, 2 *N* ammonia at room temperature released the alanine from walls, partly as alanine amide, without simultaneous release of comparable amounts of teichoic acid. Alanine and its amide were formed similarly from the walls of *Staph. aureus* (Oxford), *albus*, *citreus* and *Streptococcus faecalis*. It follows that teichoic acid is not bound to the wall by covalent linkages involving the amino-groups of the O-alanyl ester residues.

Table 1. Extraction of Teichoic Acid and Alanine from Isolated Walls

Results are expressed as moles/mole of phosphorus originally present in the wall

	Reagent	Phosphorus in solution	Alanine in solution	Ratio alanine : phosphorus
L. arabinosus	5 per cent trichloroacetic acid, 24 hr., 28°	0·71	0·36	0·51
,,	0·1 *M* acetate, *p*H 6·5 24 hr., 28°	0·102	0·40	3·95
,,	0·1 *M* acetate, *p*H 9, 24 hr., 28°	0·15	0·61	4·0
,,	2 *M* ammonia, 3 hr., 18°	0·038	0·37	9·7
,, (pre-treated with ammonia)	5 per cent trichloroacetic acid, 24 hr., 28°	0·44	0·02	0·05
B. subtilis	5 per cent trichloroacetic acid, 24 hr., 28°	0·88	0·36	0·41
,,	Water, 3 hr., 18°	0·023	0·02	0·91
,,	2 *M* ammonia, 3 hr., 18°	0·039	0·29	7·4
Staph. aureus (Duncan)	5 per cent trichloroacetic acid, 24 hr., 28°	0·67	0·55	0·82
,,	Water, 3 hr., 18°	0·059	0·055	0·93
,,	2 *M* ammonia, 3 hr., 18°	0·121	0·60	5·0

This conclusion has been confirmed for *L. arabinosus*, *B. subtilis* and *Staph. aureus* (Duncan) by treatment of their walls with fluorodinitrobenzene. The results are shown in Table 2. The reaction is heterogeneous in these experiments and thus is not necessarily complete, particularly as the walls studied may differ in their permeability towards the reagent. It is noteworthy that since reaction is usually effected at *p*H 7·8 or even *p*H 9 for several hours, a considerable proportion of alanyl ester groups would be expected to hydrolyse, liberating alanine or dinitrophenylalanine into solution. This was observed in the cases examined here. Thus, in the examination of terminal groups in bacterial cell walls which contain labile amino-acid residues, it is essential to examine the supernatant solution as well as the dinitrophenyl-walls, a procedure which, in the past, has not been followed[11].

Table 2. Dinitrophenylalanine formed on Treatment of Walls with Fluorodinitrobenzene

Conditions used were similar to those described by Salton (ref. 11). Results are expressed as moles dinitrophenylalanine/mole phosphorus originally present in walls

	Wall hydrolysate	Supernatant and water washings	Total
L. arabinosus	0·35	0·17	0·52
B. subtilis	0·17	0·11	0·28
Staph. aureus	0·32	0·18	0·50

The ribitol teichoic acids examined in detail (from *B. subtilis*[10] and *Staph. aureus*[5]) are polymers in which glycosylribitol units are joined together in a regular manner through phosphodiester groups at the 1- and 5-positions in the ribitol. Moreover, each polymer contains only one phosphomonoester group, which is situated exclusively at the 5- position of a terminal glycosylribitol unit. This has also been observed in the teichoic acid from *L. arabinosus* (unpublished observation). It follows that these compounds cannot be attached to the bacterial wall through phosphotriester linkages, as the fission of such linkages would result in the appearance of some 2,5-phosphodiester linkages or of some terminal phosphomonoester groups at positions 1 and 2 in the ribitol. Some polymer lacking a terminal phosphomonoester group would also be expected. These consequences are apparent from work in the nucleotide field[12]. Phosphotriester linkages of the type required would be extremely labile towards alkali, and this is inconsistent with the observed rate of liberation of teichoic acids from walls under alkaline conditions.

Thus, in the native walls the teichoic acid has free phosphate and amino-groups, and is presumably held in the wall through ionic linkages and hydrogen bonds. This would result in the walls bearing a charge, a conclusion supported by several observations. The ease of centrifugation of walls under various conditions can be explained on this basis, native walls being much more readily sedimented from dilute salt or trichloroacetic acid than from water. The ease of sedimentation of extracted walls in water is much greater than that of unextracted walls, and is not appreciably different from that of suspensions in dilute salt solutions. Moreover, in electron microscopical examination of natural and extracted walls we have observed repeatedly that, whereas in shadowed specimens and in stained sections native walls are well dispersed, extracted walls show a marked tendency to aggregate. These properties could arise through mutual electrostatic repulsion of the unextracted walls, this repulsive force being reduced after removal of the teichoic acid.

The ionic nature of teichoic acids in walls is consistent with the view that these compounds may regulate the passage of ions between the organism and its environment. It is perhaps significant that *Lactobacilli*, many of which contain teichoic acids in their walls, can grow in acid solutions and in the presence of fairly high (4 per cent) salt concentrations; similarly. *Staph. aureus*, *albus* and *citreus* grow in 10 per cent sodium chloride, *B. subtilis* in 7–12 per cent and *Strep. faecalis* in 6·5 per cent sodium chloride.

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[1] Salton, M. R. J., in *The Bacteria*, **1**, edit. by Gunsalus, I. C., and Stanier, R. Y. (Academic Press, New York and London, 1960).
[2] Ikawa, M., *J. Biol. Chem.*, **236**, 1087 (1961).
[3] Tokuyasu, K., and Yamada, E., *J. Biophys. Biochem. Cytol.*, **5**, 123 (1959).
[4] Archibald, A. R., Baddiley, J., and Buchanan, J. G., *Biochem. J.* (in the press).
[5] RajBhandary, U. L. (unpublished observations).
[6] Park, J. T., and Hancock, R., *J. Gen. Microbiol.*, **22**, 249 (1960).
[7] Rogers, H. J., and Perkins, H. R., *Nature*, **184**, 520 (1959).
[8] Work, E. (personal communication).
[9] Armstrong, J. J., Baddiley, J., Buchanan, J. G., Carss, B., and Greenberg, G. R., *J. Chem. Soc.*, 4344 (1958).
[10] Armstrong, J. J., Baddiley, J., and Buchanan, J. G., *Biochem. J.*, **76**, 610 (1960).
[11] Salton, M. R. J., *Biochim. Biophys. Acta*, **24**, 9 (1957).
[12] Brown, D. M., Magrath, D. I., and Todd, A. R., *J. Chem. Soc.*, 4396 (1955).

MECHANISM OF ACTION OF ANTIGEN-ANTIBODY COMPLEXES: THEIR EFFECT ON MAST CELLS

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RECENTLY, Germuth and McKinnon[1] have made the important observation that soluble antigen-antibody complexes prepared in excess antigen produce anaphylactic shock when injected into unsensitized guinea pigs. A syndrome similar to anaphylaxis was also obtained by injection of soluble complexes in mice by Tokuda and Weiser[2] and in rats by Arocha and McIlreath[3]. The observation that in the above-mentioned animals the character of the shock induced by soluble antigen-antibody complexes was very similar to that seen in anaphylaxis has suggested that the sites of antigen-antibody action are the same regardless of whether anaphylaxis is induced by injection of antigen into an already-sensitized animal or by injection of soluble preformed antigen-antibody complexes. It is known that mast cell damage and histamine release occur in guinea pig active or passive anaphylaxis[4–6], as well as in rat and mouse active anaphylaxis[7–10], so I thought it would be interesting to find out whether soluble antigen-antibody complexes would duplicate damage to mast cells and release of histamine observed in anaphylaxis.

Crystalline bovine serum albumin was used as antigen. The antiserum was a pool of rabbit anti-bovine serum albumin containing 1·0 mgm. antibody nitrogen/ml.

To prepare the soluble complexes antigen was added to antiserum at equivalence, the mixture incubated at 37° C. for ½ hr. and kept for 24 hr. at 4° C. The precipitate was then washed three times with saline at 0–1° C., re-suspended in Tyrode solution containing a total amount of bovine serum albumin ranging from 5 to 8 times that used to precipitate antibody at equivalence, stored at 4° C. with occasional agitation for 48 hr. and cleared by centrifugation.

Twenty guinea pigs each weighing 250 gm. were injected intravenously with 2 ml. (2 mgm. antibody nitrogen) of the complexes preparation. Three of these guinea pigs died with emphysematous lungs, 10 presented severe shock and died overnight, 2 presented slight to moderate shock and 5 presented no effect. The emphysematous lungs were then fixed and stained for microscopical observation of the mast cells as described elsewhere[4]. However, a microscopical inspection of many fields did not reveal any mast cell degranulation. Nevertheless, it was considered that the amount of histamine released, although enough to induce emphysema, was insufficient to produce any visible mast cell damage and it was decided to perform *in vitro* experiments, in which the effect of the complexes on the mast cells could be detected more easily. Thus, 15 guinea pigs were killed by breaking the neck, their lungs, ileum and mesentery dissected out, and the activity of the complexes tried on these organs. The ileum was suspended in a Dale apparatus with 7 ml. capacity and the activity of preparations of the complexes tested by adding 0·4 ml. (400 μgm. antibody nitrogen) of them to the bath. The lung was cut in slices 0·8 mm. thick, washed in cold Tyrode solution, and 10–12 of these slices used as samples. They were then shaken in beakers closed with 'Parafilm' caps for 15 min. at 37° C. in 4 ml. (4 mgm. antibody nitrogen) of preparations of the complexes or in 4 ml. of Tyrode solution, after which the histamine content of the supernatants was assayed on the guinea pig ileum. The mesentery was cut into pieces which were incubated with the complexes under the conditions described for the lung, and then transferred to a 4 per cent solution of lead subacetate for fixation[4].

Six of the 15 ilea tested responded to the complexes with a definite contraction followed by desensitization as already reported[11]. However, no release of histamine or damage to mast cells could be detected when the lungs or mesentery of the same animals were incubated with the complexes. The same results were obtained when 1 per cent fresh guinea pig serum was added to the complexes. Twenty rats were injected intravenously with 2 ml. (2 mgm. antibody nitrogen) of the preparation of complexes. All the animals presented moderate to severe shock, but there were no fatalities. It was then observed that adrenalectomized rats were very susceptible to the effect of soluble antigen-antibody complexes and 15 rats adrenalectomized 48 hr. before were injected intravenously with 1 ml. (1 mgm. antibody nitrogen) of the complexes. All the animals showed severe shock and 12 died showing generalized intestinal congestion at autopsy. For microscopical observation of the mast cells, the skins of the snout and the tongue were fixed by injecting fixative through the carotid artery as described elsewhere[8]. However, no damage to

the mast cells was observed, except for one rat in which there was generalized damage of these cells. This experiment was duplicated with a group of 12 adrenalectomized rats from which blood was collected 5 min. after injection of the complexes and the plasma histamine assayed on the ileum. Although all the animals showed severe shock no increase in plasma histamine was detected, except for one animal in which 0·5 μgm./ml. of histamine was found. Twenty mice were then injected with 0·5 ml. (0·5 mgm. antibody nitrogen) of the complexes. All the animals showed moderate to severe shock, and 9 of them died within 10 min. However, microscopical examination of their tissues did not reveal any damage to the mast cells. Pretreatment with *B. pertussis* aggravates the shock induced by soluble antigen-antibody complexes in mice[2] and 20 *pertussis*-treated mice were injected with 0·5 ml. of soluble complexes. Although 18 of these mice have presented severe shock and died within 20 min. no damage to the mast cells could be observed in their tissues.

These results showing that guinea pigs, rats and mice injected with antigen-antibody complexes in excess antigen present an anaphylactic picture similar to that observed in anaphylaxis confirm the results of several authors[1–3]. However, I have not been able to duplicate by means of soluble antigen-antibody complexes the damage to mast cells and release of histamine observed in anaphylaxis.

I consider these results show that neither release of histamine nor damage to mast cells occurs when guinea pig lung or mesentery is incubated with soluble antigen-antibody complexes, under conditions in which both damage to mast cells and release of histamine are promptly produced when antigen contacts sensitized tissues[5]. Furthermore, my results suggest that the complexes do not act as a histamine releaser or by sensitizing the tissues and reproducing passive anaphylaxis. Also the possibility that anaphylatoxin could have been formed by reaction between antigen-antibody complexes and serum proteins is ruled out as anaphylatoxin induces damage to mast cells similar to that produced by antigen-antibody reaction in anaphylaxis[12]. These results certainly do not exclude that soluble antigen-antibody complexes may be operative in anaphylaxis, but suggest that the complexes are not able, or at least not quite efficient, in duplicating the cytological aspects of anaphylaxis connected with liberation of histamine. However, that this may be possible under certain conditions is suggested by my observation that some of the adrenalectomized rats show damage to mast cells and release of histamine when injected with soluble complexes. This same fact, or a possible difference in the composition of the complexes used, may explain the results of Arocha and McIlreath[3], who have reported release of histamine from the isolated lung of the guinea pig or small intestine of the rat when perfused with soluble antigen-antibody complexes. However, the experiments reported here suggest strongly that soluble antigen-antibody complexes do not preferentially act by releasing histamine from the mast cells.

[1] Germuth, F. G., and McKinnon, G. E., *Bull. Johns Hopkins Hosp.*, **101**, 13 (157).
[2] Tokuda, S., and Weiser, R. S., *Science*, **127**, 1237 (1958).
[3] Arocha, H. G., and McIlreath, F. J., Twenty-first Intern. Cong. Physiol. Sc., Buenos Aires (1959).
[4] Mota, I., and Vugman, I., *Nature*, **177**, 427 (1956).
[5] Mota, I., *J. Physiol.*, **147**, 425 (1959).
[6] Humphrey, J. H., and Mota, I., *Immunol.*, **2**, 31 (1959).
[7] Mota, I., Thesis, Univ. of São Paulo (1953).
[8] Mota, I., *Brit. J. Pharmacol.*, **12**, 453 (1957).
[9] Mota, I., *Nature*, **182**, 1021 (1958).
[10] Carter, P. B., Higginbotham, R. D., and Dougherty, R. D., *J. Immunol.*, **79**, 259 (1957).
[11] Trapani, I. L., Gravey, J. S., and Campbell, D. H., *Science*, **127**, 700 (1958).
[12] Mota, I., *Immunol.*, **2**, 403 (1959).

CHROMATOGRAPHIC ANALYSIS OF INFECTIOUS RIBONUCLEIC ACID FROM POLIOVIRUS

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TWO metabolically distinct types of ribonucleic acids (RNA) have been recognized in mammalian and bacterial cells: a low molecular weight or soluble component (LMW-RNA) which is apparently free in the cytoplasm[1] and able to transfer the activated amino-acids to the ribosomes[2], and a highly polymerized form or high molecular weight component (HMW-RNA) linked to a protein moiety in a ribonucleoprotein structure (RNP, ribosomes) which is regarded as the major site of protein synthesis[3]. Another type of RNA is that of the mature particles of some plant and animal viruses (viral RNA), which seems able to direct the synthesis of the whole virus by a suitable host cell. Numerous publications have, in fact, reported the infectivity of naked RNA extracted with cold[4,5] or hot[6] phenol from different viral particles.

The system of cells infected with viral RNA from poliovirus presents a particular difficulty, because once extracted with phenol, most of the protein-free polio-RNA apparently remains no longer infectious[7]: a decrease of 3–5 log units is usually observed in different experiments, an upper limit of 1 per cent residual infectivity being only recently reported[8].

Some experiments of partition chromatography described in the present article were carried out to examine the relationships between cellular and viral RNA's, and to analyse the infectivity of protein-free RNA from poliovirus with a possible exclusion of cellular constituents. Our work is based on the use of a chromatographic column (protein-coated 'Supercel') which can separate the high molecular weight RNA (found by us only in the ribosomes) from the low molecular weight RNA (found mainly, but not only, in the supernatant on centrifugation of bacterial homogenates at 100,000*g*) and from the precursors

contained in the phenol extracts of *E. coli*[9,10]. Such a column has also been used for research on deoxyribonucleic acids (DNA) from bacteriophages[9] as well as for the analysis of RNA metabolism in phage-infected bacteria[10] and of HeLa cells infected with different DNA- and RNA-viruses[11], because it allows separation of small amounts of nucleic acids without the necessity for previous isolation from large quantities of contaminating precursors. The column comprises analytical grade cellulose powder, protein-coated 'Supercel', 'Supercel' coated with albumin made basic by methylation and washed with 0·3 *M* buffered sodium chloride, 'Supercel'. Procedure of methylation and details for preparation of the column are to be found in refs. 9–14.

HeLa cells employed throughout this work were cultured in suspension in spinner flasks incubated at 37° C., using as growth medium Hank's solution lacking calcium and magnesium but supplemented with ten times as much phosphorus and with 5 per cent decomplemented calf serum and 0·5 per cent lactalbumin hydrolysate. Medium 199 added with carrier-free preparations of phosphorus-32 labelled orthophosphate to a specific activity of 1 or 2 μc. phosphorus-32/μgm. phosphorus-31 was used to yield radioactive cells. Virus stocks were prepared by infecting monolayers of either HeLa cells or monkey kidney primary cultures with multiplicities 1–5 of poliovirus type *I*, allowing an incubation of 20 hr. at 37° C. When labelled virus had to be obtained, after the contact with the virus, monolayers were washed with a phosphorus-free Hank's solution buffered at *p*H 7·6 with 0·01 *M tris* and then covered with medium 199 added with phosphorus-32 labelled inorganic phosphate. After lysis had occurred, cellular debris was spun down by low-speed centrifugation: spontaneous lysates thus obtained had the usual titre of 1×10^7–5×10^7 plaque-forming units (pfu)/ml. The virus was concentrated either by centrifugation at 100,000*g* for 2 hr., or by precipitation at *p*H 3·5 and 3° C. with hydrochloric acid and yeast-RNA as carrier: with the first method up to 10^5 pfu/ml. and with the second 10^8 pfu/ml. remained in the supernatant. Virus suspensions of high titre were also obtained by sonicating at 3° C. cells collected by centrifugation 10 hr. after infection with poliovirus, but these preparations were heavily contaminated by cellular RNA's and ribosomes. A further purification of the concentrated virus preparation could be achieved through a treatment with RNase at 37° C. followed by ultracentrifugation in the cold to remove the enzyme. Titrations of viable virus were usually performed on monolayers of trypsin-dispersed monkey kidney cells, following Dulbecco and Vogt's plaque-technique[15].

For the extraction of cellular RNA's, HeLa cells washed free from the radioactivity of the medium and suspended in 0·1 *M* sodium chloride buffered at *p*H 7·1 with 0·1 *M* sodium phosphates were extracted three times with water-saturated redistilled phenol at 60° C. and constant stirring: such procedure, however, proved to be unsuitable for the extraction of poliovirus, since a loss of the whole viral infectivity occurred when temperature exceeded 40° C. during the phenol treatment. For the preparation of viral RNA, concentrated suspensions of virus in the same buffered saline were extracted three times with phenol at 3° C.; residual phenol was removed by five extractions with peroxide-free ether and the latter eliminated by bubbling a stream of sterile nitrogen[10]. Preparations of cellular and viral RNA's were eluted from a protein-coated 'Supercel' column with a linear gradient of sodium chloride 0·3 → 1·5 *M* constantly buffered at *p*H 6·7 with 0·05 *M* phosphates[10]. The adsorption at 254 mμ and radioactivity of the eluate were continuously measured with ultra-violet adsorption and count-rate meters, and simultaneously recorded: measures with ultra-violet optics were omitted when infectivity of viral RNA had to be titrated on monkey kidney monolayers[15]. Molarity of a given fraction was obtained from the formula $C = C_. + C_f(e^{x/v} - 1)e^{-x/v}$, where $C_.$ = concentration of the mixer; C_f = concentration of the feeder; x = volume delivered less column displacement; v = vol. of the mixing vessel. A step-wise elution was also carried out with solutions 0·3 *M*, 0·5 *M*, 0·7 *M*, 1·0 *M* and 1·5 *M* sodium chloride buffered at *p*H 6·7, in which case the whole infectivity was eluted at 1·0 *M*. The optical density of the single fractions was measured in a Unicam spectrophotometer and the radioactivity with a gas-flow counter.

Fig. 1*b* shows the elution pattern of phosphorus-32 labelled compounds from HeLa cells: similarly to that observed with *E. coli*[10], peak *A* contains free bases, nucleotides and optically inactive phosphorus-32 labelled compounds (precursors), peak *B*, RNA of low molecular weight (LMW-RNA), and peak *D*,

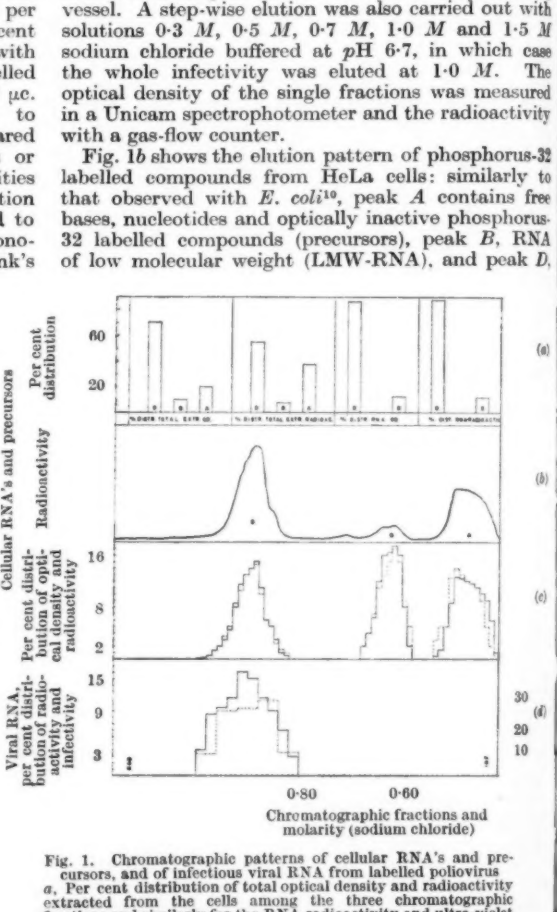

Fig. 1. Chromatographic patterns of cellular RNA's and precursors, and of infectious viral RNA from labelled poliovirus *a*, Per cent distribution of total optical density and radioactivity extracted from the cells among the three chromatographic fractions and similarly for the RNA-radioactivity and ultra-violet adsorption; *b*, elution pattern of phosphorus-32 labelled compounds extracted with phenol from HeLa cells grown for four generations in the presence of phosphorus-32 labelled orthophosphate. Peaks *B* and *D* represent cellular RNA's; precursors are contained in the fraction *A*; *c*, per cent distribution of optical density at 254 mμ (dashed lines) and radioactivity (continuous lines) among the three chromatographic peaks. Total ultra-violet adsorption and radioactivity within each peak considered equal to 100; *d*, per cent distribution of radioactivity (continuous lines) and infectivity (dashed lines) of viral RNA extracted with cold phenol from phosphorus-32 labelled poliovirus prepared on monkey kidney cell monolayers

of high molecular weight (HMW-RNA). The peak *C* of DNA, which is located between the two RNA-regions, does not appear because of the DNA-exclusion during extraction with hot phenol; it appears, however, when extraction is carried out at a lower temperature, that is, at 4°–40° C., and on addition of detergent, namely, sodium dodecyl sulphate 0·5–4 per cent, in which case the repartition of radioactivity among the three nucleic acid fractions is the following: $B : C : D = 8 : 32 : 60$. Figs. 1*a* and 1*c* show the distribution (per cent) of both ultra-violet adsorption and radioactivity among the three chromatographic fractions. Within the same area of (high molecular weight) RNA from the host cell, viral RNA is also eluted, as shown in Fig. 1*d*, in which the distribution of both radioactivity and infectivity for monkey kidney cell monolayers are reported (practically, 100 per cent of the viral RNA infectivity loaded on the column was recovered within this peak).

These experiments indicate that viral RNA from poliovirus behaves chromatographically in a similar way to cellular HMW-RNA, but quite differently from LMW-RNA. Differences between the two types of cellular RNA's in relation to the method of partition chromatography used have been discussed[9,10]. The recovery of infectious naked RNA from poliovirus by partition chromatography indicates that the protein-coated 'Supercel' column could be a suitable means for purification and isolation of viral RNA. In addition, our results may support further the view that isolated poliovirus-RNA is fully infectious, and exclude the participation of other viral or cellular components in such infectivity, a view that is in agreement with the theory of viral RNA as carrier of genetical information, since protein-free RNA's, but not nucleoproteins, seem to be eluted from our column. We have been unable to find any difference between inactive and infectious viral RNA from poliovirus extracted with phenol, and the reason for the partial inactivation at 0° C., when compared with the complete loss occurring at 60° C.

Note added in proof. While this work was in the press, Dr. L. Philipson (Rockefeller Institute, New York) informed us that with suitable gradients two fractions of high molecular weight RNA having sedimentation coefficients of 16 and 25–29 can be separated further on the type of column we have used, and that the DNA extracted with cold phenol from HeLa cells has an $S^{\circ}_{20,w}$ of 38, corresponding to a molecular weight of 32×10^6.

[1] Tissières, A., *J. Mol. Biol.*, **1**, 365 (1959).
[2] Zamecnik, P. C., Stephenson, M. L., and Scott, J. F., *Proc. U.S. Nat. Acad. Sci.*, **46**, 811 (1960).
[3] Hoagland, M. B., Zamecnik, P. C., and Stephenson, M. L., *Biochim. Biophys. Acta*, **24**, 215 (1957).
[4] Gierer, A., and Schramm, G., *Z. Naturforsch.*, **11b**, 138 (1956).
[5] Fraenkel-Conrat, H., Singer, B., and Williams, R. C., *Biochim. Biophys. Acta*, **25**, 87 (1957).
[6] Wecker, E., *Virology*, **7**, 241 (1959).
[7] Prinzie, A., Schonne, E., and De Somer, P., *Arch. Virusforsch.*, **10**, 153 (1960).
[8] Koch, G., Koenig, S., and Alexander, H. E., *Virology*, **10**, 329 (1960).
[9] Mandell, J. D., and Hershey, A. D., *Anal. Biochem.* (in the press).
[10] Cocito, C., *Proc. Intern. Biophys. Cong.*, Stockholm (August 1961).
[11] Cocito, C., Prinzie, A., and De Somer, P., *Proc. Intern. Cong. Biochem.*, Moscow (August 1961). Cocito, C., and De Somer, P., *Proc. Intern. Biophys. Cong.*, Stockholm (August 1961). Cocito, C., Prinzie, A., and De Somer, P., *Biochim. Biophys. Acta* (in the press).
[12] Fraenkel-Conrat, H., and Olcott, H. S., *J. Biol. Chem.*, **161**, 259 (1945).
[13] Lerman, L. S., *Biochim. Biophys. Acta*, **18**, 132 (1955).
[14] Brown, G. L., and Watson, M., *Nature*, **172**, 339 (1953).
[15] Dulbecco, R., and Vogt, M., *J. Exp. Med.*, **99**, 167 (1954).

BLOOD-FEEDING, FECUNDITY AND AGEING IN *AEDES AEGYPTI* VAR. *QUEENSLANDENSIS*

By Dr. M. M. J. LAVOIPIERRE

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IN *Aedes aegypti*, as in many other culicines, a blood meal is essential for the maturation of the eggs, but it does not appear to be necessary for survival, since, under experimental conditions, this mosquito will live for many weeks on simple carbohydrates, notably certain hexoses and disaccharides[1]. This is not peculiar to mosquitoes, for in a wide range of biting and non-biting Diptera the normal life-span is attained on a carbohydrate diet[2]; the protein meal, apparently, is basically related to reproduction.

Adult female *A. aegypti* var. *queenslandensis* reared from well-nourished larvæ usually require only a single blood meal for the maturation of their oocytes[3], the number of eggs which develop depending on the host from which the meal is obtained[4]. After the first blood meal mated females may bite again, but their avidity for blood diminishes markedly as the eggs mature, biting being minimal just before oviposition[5]. After egg-laying, avidity once again becomes pronounced, and the cycle is repeated. On the other hand, in the case of the unmated insects, biting activity is high throughout the gonadotrophic cycle, although the number of eggs maturing and the speed at which they develop is no less than that of the mated flies. The unmated females retain most of the mature eggs during the remainder of their lives, deposition of the small number laid being extended over many weeks[6]; none the less, avidity for blood continues to be pronounced for several weeks, diminishing gradually as the population ages.

Since these results were published, I have shown that the blood-feeding drive is apparently closely related to oogenesis and to vitello-genesis. Supernumerary blood meals taken by unmated females were abnormally metabolized, producing, particularly in the ovary, aberrant changes which were especially noticeable in older flies. Moreover, the protein meal shortened the life-span of the unmated mosquitoes: a comparison (Figs. 1 and 2) of the survival-curves of mated and unmated insects, offered daily meals on human subjects, shows that the mated flies survived much longer than those which were unmated. The increase in death-rate

Table 1. 50 per cent mortality and life-expectancy of, and the number of eggs (calculated as a percentage) oviposited by, several populations of (*a*) mated and (*b*) unmated females of *Aedes aegypti* var. *queenslandensis* offered blood meals (for 10 min.), daily and at intervals, over a period of many weeks. In the mated group three cages were set up, each with a random number of mosquitoes—individuals which had not fed when the first blood meal was offered (the numbers are indicated in brackets) were removed and discarded; in the unmated series 140 previously fed mosquitoes were divided into two equal groups

Whether mated or unmated	Frequency of feeding	No. of mosquitoes	50 per cent mortality in days	Life-expectancy in days after 1st blood meal	Further life-expectancy in days at age 35 days	No. of eggs laid at 50 per cent mortality (calculated as a percentage)
Mated	Daily	104 (3)	69·0	65·1	35·5	44,166
	Every 8 days	85 (12)	81·0	77·0	46·5	33,761
	Every 16 days	62 (10)	91·0	80·9	53·0	26,613
Unmated	Daily	70	38·5	38·6	11·4	1,949
	Every 16 days	70	39·0	40·3	14·3	1,820

seems related to the metabolism of the blood meal, since virgin females of *A. aegypti* var. *queenslandensis* slightly outlive mated females when the populations are maintained on a sucrose diet alone[7]. In the case of certain other insects, however, for example, *Drosophila obscura* and *Periplaneta americana*, which discriminate much less than *A. aegypti* in their food-seeking, a diet which is responsible for high fecundity in mated females does not appear to reduce the life-span of the unmated females, which, indeed, live longer than their mated fellows[8].

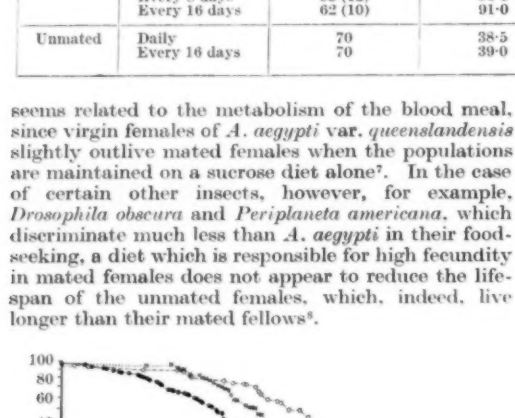

Fig. 1. Survival curves for comparable groups of mated *Aedes aegypti* var. *queenslandensis* offered blood meals daily ●, every 8 days ×, and every 16 days ○, plotted on semilogarithmic co-ordinates. The three groups were offered the initial meal on the same day

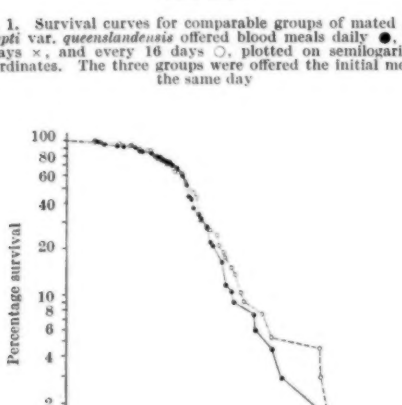

Fig. 2. Survival curves for comparable groups of unmated *Aedes aegypti* var. *queenslandensis* offered blood meals daily ●, and every 16 days ○, plotted on semilogarithmic co-ordinates. The two groups were offered the initial meal on the same day

Decreasing the frequency of feeding apparently scarcely influences the death-rate of the unmated females. The survival curves of two comparable populations, one fed daily and the other every sixteenth day, were found to approximate very closely (Fig. 2): calculations for life-expectancy differed little, a *t*-test giving $P > 0{\cdot}1$ and $< 0{\cdot}2$. In both populations the number of eggs deposited at 50 per cent mortality was small and almost the same (Table 1).

When, however, three comparable groups of mated mosquitoes were offered blood meals, daily, every eighth day, or every sixteenth day, the results were entirely different. The death-rate of the mated insects increased significantly with increased frequency of feeding, a *t*-test applied to the daily and 16-day groups giving $P = 0{\cdot}001$ (Table 1, Fig. 1). Associated with the more frequent opportunities to feed was a definite augmentation in the number of eggs laid, the daily-fed group laying 27 and 66 per cent more eggs than the eighth- and the sixteenth-day groups respectively at 50 per cent mortality.

These observations appear to indicate that while increased frequency of blood-feeding in mated females of *A. aegypti* var. *queenslandensis* allowed to lay eggs was accompanied by a marked increase in the death-rate of the mosquitoes, the effect seemed to be associated with augmentation in fecundity alone, for, in unmated females, which laid few eggs, increased feeding had no significant effect on the death-rate.

The results also suggest that the number of eggs laid by the mosquito is a parameter which may be of value when the probability of survival of mosquito populations both in the laboratory and in Nature is computed. The number of eggs laid depends on the number of ovipositions, and since the number of times a mosquito has oviposited can now be determined[9], the findings reported in this article may be of practical significance to investigators working on the dynamics of field populations of mosquito vectors of disease.

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[1] Galun, R., and Fraenkel, G., *J. Cell. Comp. Physiol.*, **50**, 1 (1957).
[2] Downes, J. A., *Ann. Rev. Entom.*, **3**, 249 (1958).
[3] Macdonald, W. W., *Ann. Trop. Med. Parasit.*, **50**, 399 (1956).
[4] Pena, E., and Lavoipierre, M. M. J., *Rev. ibér. Parasit.*, **20**, 23 (1960).
[5] Lavoipierre, M. M. J., *Nature*, **181**, 1781 (1958).
[6] Pena, E., and Lavoipierre, M. M. J., *Rev. ibér. Parasit.*, **20**, 163 (1960).
[7] Pena, E., and Lavoipierre, M. M. J., *Rev. ibér. Parasit.*, **20**, 39 (1960).
[8] Maynard Smith, J., *J. Exp. Biol.*, **35**, 832 (1958).
[9] Macdonald, G., Detinova, T. S., and Bertram, D. S., *W.H.O. Mal.* 238 (1959).

BRAIN WAVES AND 'BLOOD WAVES'

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IN search of an efficient method for localizing brain tumours, the temperature records in Fig. 1 were obtained from a normal female head with a modified Beckman d.c. breaker amplifier, a Leeds and Northrup paper recorder, and a copper-constantan thermocouple. The broken-line curve was taken with the thermocouple attached to the right temple and the solid line with it attached to the middle of the forehead about 4 cm. above the glabella. This latter record was obtained 24 hr. after the first. The subject sat comfortably in an arm-chair and was asked to relax with her eyes shut. At the first points *O*, about 5 min. later (the first 4 min. of the record is not shown in Fig. 1, the points *O* are shown at 0·6 min. later) the subject was asked to open her eyes and was immediately handed a multiplication problem. She finished multiplying a five-digit number by a four-digit number at the points *F* some 2 min. later. In this interval a large temperature change was noted. At points *C* she was asked to close her eyes and completely relax, and at the second points *O* was told to open them again. However, on the solid-line curve at point *O* she was given a second multiplication problem, whereas on the broken-line curve was engaged in a spirited conversation. From points *C* to *O* a temperature drop of about 3° occurred.

After examining the curves, it was decided that these variations in temperature or 'heat waves' were due to a change in blood-flow in these regions. Since it is known that problem solving is accompanied by fast electroencephalographic (EEG) waves[1] it was thought that these brain waves may be variations in potential between sites due to ions in motion in the blood vessels. That is, brain waves may actually be principally 'blood waves'. To test this electrohæmodynamic hypothesis a model was constructed as shown in Fig. 2, to simulate a simple blood vessel system. A 3-l. glass bottle filled with physiological saline was connected by 5 mm. inside diameter (all diameter dimensions given are inside diameter unless otherwise indicated) rubber tubing to a piece of polyethylene tubing 10 cm. long with a diameter of 2 cm. A hypodermic needle stylet *C* was forced

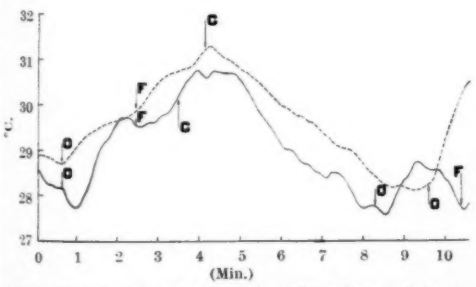

Fig. 1. Temperature variations on the surface of a normal female head during experiment. The broken line curve taken on the right temple and solid line on the forehead 4 cm. above the glabella

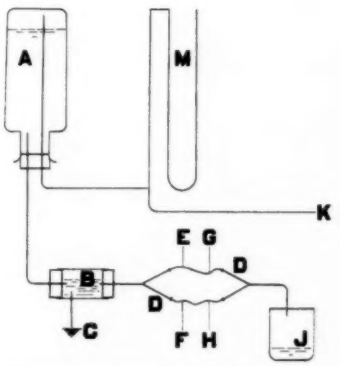

Fig. 2. Schematic representation of simulated simple blood vessel system

half-way into this large tube in contact with the saline solution to serve as an EEG reference point or earth. The tube *B* was then connected to a glass *Y* connector *D*, and the two arms were each connected to No. 18 hypodermic needles. A duplicate of *D* connected to vessel *J* was coupled to it by two 20-cm. pieces of No. 18 (approximately 1 mm. diameter) polyethylene tubing via the No. 18 needles. Four size 35 suture wires (*E*, *F*, *G* and *H*) were forced into this small tubing 7·5 cm. apart to make contact with the saline solution. Wires *E* and *F* were connected to a flashlight battery to simulate d.c. potentials that may be present at a site. Wires *G* and *H* were connected to an EEG channel. The rubber tubing leading to the receptacle *J* had a diameter of 3 mm. The manometer *M* and the 3-mm. tubing *K* were connected by a glass *T* to bottle *A*. Whenever *K* was left open the solution in *A* was at zero pressure (or atmospheric pressure) and pressure and vacuum variations were applied by inhaling or exhaling into this opening. The records shown in Fig. 3 were obtained from this system.

At atmospheric pressure the normal flow of the saline solution from bottle *A* through the system and finally into the container *J* was 60 c.c./min. The recording speed was 30 mm./sec. and each recording in Fig. 3 was obtained successively in a single experiment. Record *A* was obtained by creating a 20-mm. vacuum in bottle *A* and pinching off the opening at *K* with a clamp. This record occurred 144 sec. after the application of the vacuum. At this time the vacuum increased to 40 mm. due to the saline solution running out. It was observed that the waves at 3 per sec. are due to the formation and severing of drops at the end of the rubber tubing in container *J*. From an initial rate of about 6 drops/sec. and amplitude of 40 μV. the flow fell to 3 drops/sec. and the amplitude increased to 180 μV. This record might be compared with a left and right parietal petit mal record of a 9·5-year-old male child[2]. Record *B* is a

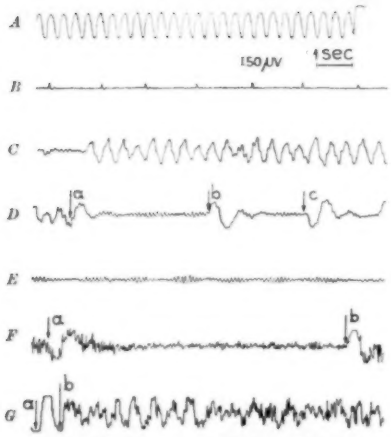

Fig. 3. Records obtained from simulated simple blood vessel system. Consecutive records from a single experiment

simulated heart beat which was obtained without a d.c. potential between *E* and *F* in Fig. 2. In this and later records the tube in container *J* was submerged below the level of the solution in *J*. The pressure at *K* was alternately built up to 30 mm. and suddenly released at intervals indicated by the distance between the spikes. When the pressure was alternately increased to 40 mm. no increase in amplitude of the spikes occurred, but remained at about 35 μV. Record *C* was obtained by squeezing the tube *K* about twice per sec. with the thumb and finger while it was clamped and the vacuum in *A* had slowly built up to 40 mm. Preceding this record there was a predominance of 10/sec. wave-forms with an amplitude of about 25 μV. while the vacuum was slowly building up. Occasionally big slow spikes and slow waves of 2–3 per sec. would appear. Amplitude modulated 9/sec. wave-forms such as in record *E* began appearing at about 33-mm. vacuum which occur in practice in the occipital region normally at any age. Record *C* can be compared with that of an 8-year-old boy in very deep sleep[2]. Record *D* occurred about 100 sec. before record *E* and the slow waves were produced by squeezing tube *K* while maintaining a 40-mm. vacuum. This record followed *C* by 20 sec., and the tube was squeezed with thumb and finger at point *a*, released at *b*, and again squeezed at *c*. Such slow waves apparently occur during light sleep of an adolescent. Record *F* was obtained by applying a pressure of 20 mm., which was released at point *a* and applied again at *b*. This recording is similar to an attention record where the eyes are closed and later opened. Recording *G* was made by first applying pressure rhythmically at about 1·5 times per sec. to point *b* (only one wave-form at this rate is included in the figure) and suddenly changing the rate at point *b* to twice per sec. These waves are similar to the paroxysmal waves in petit mal[3].

It is interesting that the heart-beat was reproduced without a d.c. potential and the other records with a d.c. potential applied. I think that these phenomena should be investigated further *in vitro* and *in vivo*, since it may explain the apparent discrepancies that occur when EEG patterns are compared with the location of certain brain lesions[4], their absence in hypothermia[5], and their presence over the area of a hemispherectomy, as I have observed.

[1] Adrian, E. D., and Matthews, B. H. C., *Brain*, **57**, 355 (1934).
[2] Gibbs, F. A., and Gibbs, E. A., *Atlas of Electroencephalography*, 1 (Addison-Wesley Press, Inc., Cambridge, 1950).
[3] Gibbs, F. A., and Gibbs, E. A., *Atlas of Electroencephalography*, 2 (Addison-Wesley Press, Inc., Cambridge, 1952).
[4] Kahn, E. A., Bassett, R. C., Schneider, R. C., and Crosby, E. C., *Correlative Neurosurgery*, 41 (C. C. Thomas, Pub., Springfield, Illinois, 1955).
[5] Callaghan, J. C., McQueen, D. A., Scott, J. W., and Bigelow, W. G., *A.M.A. Arch. Surgery*, **68**, 208 (1954).

FACTS AND THEORIES OF SHAPE DISCRIMINATION

IN a recent article[1] Sutherland attempted to evaluate three theories of shape discrimination (actually, they are theories of the coding of information about shape in the brain, none is a complete theory as it stands.) The three theories are: Sutherland's own theory, which he labels theory *A* (ref. 2); a recent theory of Deutsch's, labelled theory *B* (ref. 3); a theory of mine, labelled theory *C* (ref. 4).

In his evaluation of these theories, Sutherland makes some incorrect statements about theories *B* and *C*, and does not mention some evidence of his own which is opposed to his own theory. The purpose of the present article is three-fold. First, to re-examine the evidence presented by Sutherland and to show that the conclusions he draws from it are unjustified; secondly, to point out several weaknesses in theory *A* which are purely logical and have nothing to do with the degree to which any one of the three theories has been verified or otherwise; and thirdly, to present some fresh observations which support theory *C*, but which do not support theory *A*. The discussion of the first topic will be confined mainly to the arguments put forward in Sutherland's article[1], and to theories *A* and *C* only, since Deutsch has already replied to Sutherland's criticisms of theory *B* (ref. 5).

Evaluation in Relation to the Various Theories

Sutherland[1] lists twelve groups of facts in his evaluation. Those facts which support both theory *A* and theory *C* will not be discussed; those which are claimed to give support to *A* but not to *C* will be discussed in the order and with the numbering given in Sutherland's article. His Fig. 1 is reproduced, together with the horizontal and vertical outputs of theory *C*, to clarify the ensuing arguments. A word about the outputs of theory *C* is in order. The two co-ordinates for an output are: on the ordinate, size of impulse; on the abscissa, time of occurrence. Thus each output is characterized by the sizes of the impulses generated and by the temporal pattern in which they occur, and either

of these properties is sufficient to make an output distinctive and discriminable. Only in cases where there is little or no difference in *both* properties for both horizontal and vertical outputs respectively does the theory predict confusion of the associated shapes. The impulses are generated by a system which sums the extent of the contours of a shape on horizontal and vertical co-ordinates over a two-dimensional array[1].

(4) *Discrimination of broken vertical rectangle.* Sutherland points out that this shape (Fig. 1*a*) will be treated as a vertical rectangle in theory *A*, which is correct; but as a horizontal rectangle in theory *C*, which is incorrect, as can be seen by comparing the

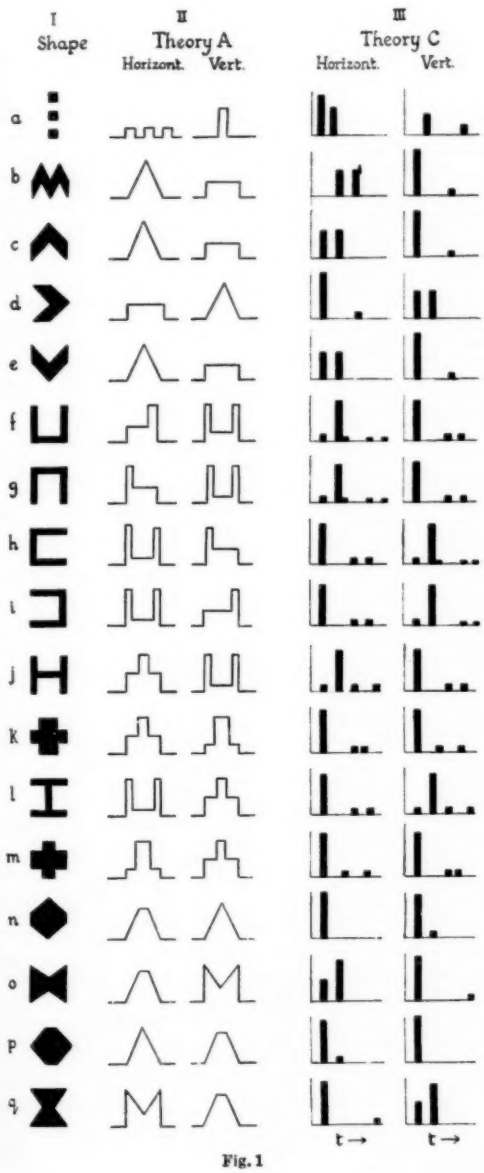

Fig. 1

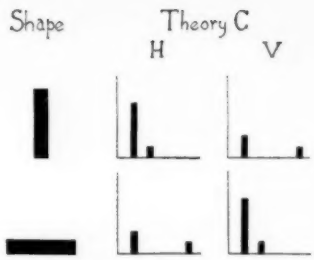

Fig. 2

outputs for this shape with the outputs for vertical and horizontal rectangles shown in Fig. 2. This suggests that Sutherland has misunderstood theory *C*. The octopus and the rat both treat the broken vertical rectangle like a vertical rectangle, but Sutherland's assertion (p. 842): ". . . This result supports *A*, but is evidence against both *B* and *C*" is plainly incorrect.

(6) *Discrimination between diamond and oblique rectangles.* Sutherland is correct in stating that these figures should be indiscriminable on *C*, and since they can be discriminated by the octopus, he considers this a crucial test of theory *C*. However, inspection of his Fig. 2*a* shows that the diamond and rectangle used in his experiment are different in area (the dimensions reported elsewhere[6] are: square 5 cm. × 5 cm., rectangle 2 cm. × 10 cm.), and since he used 'filled in' shapes, or surfaces, rather than contours, it is obvious that the discrimination could be a simple brightness discrimination. Although some of the subsequent transfer tests suggest that discrimination was not based on brightness differences alone, no direct test of the effect of the brightness difference was made. As it stands, this finding can scarcely be considered a test of theory *C*, much less a crucial test.

(8) *Discrimination between shapes b, c, d and e (Fig. 1).* Theory *A* predicts that *b* and *c* should be indiscriminable, but they are discriminable. This is evidence against theory *A*, and to save it Sutherland has to introduce an additional assumption (ref. 1, p. 844 (1)). Inspection of the outputs for theory *C* in Fig. 1 shows that: (i) *b* and *c* should be discriminable, but with difficulty; (ii) *c* and *d* should be readily discriminated; and (iii) after training on *c* and *d*, *b* should be treated like *c*. Sutherland found all these three to be true, so his evidence supports *C* rather than *A*, since all three can be predicted from *C* without introducing additional assumptions. The lack of discrimination between *c* and *e* supports *A* and *C* equally.

(10) *Discrimination between up-down and right-left mirror images.* The fact that discrimination is possible appears to refute *C*, since inspection of the outputs for the pairs of shapes *f*, *g* and *h*, *i* (for example) shows that they are identical for each pair. However, Sutherland's use of filled-in shapes again makes his mirror-image experiments a poor test of *C*. While the overall brightness of shapes *f* and *g* is the same, for example, the distribution of brightness is different. Thus this pair could be discriminated on the basis of 'brighter above' (*g*) versus 'brighter below' (*f*). This possibility should at least be examined experimentally before adducing this evidence as a ground for refuting *C*. It may be noted that where there is

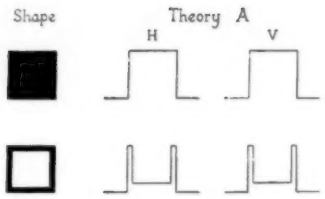

Fig. 3

not such an obvious difference in brightness distribution (as in shapes *c* and *e*), there is also no discrimination between the pair.

(12) *Discrimination of the pairs of shapes j, k; l, m; n, o; and p, q.* Inspection of the outputs for theory *C* shows that all four pairs should be easily discriminable, whereas theory *A* predicts that the pairs *j*, *k* and *n*, *o* should be less discriminable than the others, on the assumption of greater resolving power for the horizontal outputs (ref. 1, p. 843). Even if this assumption were incorporated (as it could be) in *C*, to account for the fact that horizontal rectangles of different extents are more readily discriminable by the octopus than vertical rectangles of different extents, this would still leave the pairs *j*, *k* and *l*, *m* equally discriminable, since both differ in both horizontal and vertical outputs in *C*. The same argument applies to the pairs *n*, *o* and *p*, *q*. So here are two cases where *C* yields a correct prediction, and *A* a false one, since all the pairs are discriminable. The remainder of the experimental findings are neutral, that is, equally explainable on either *A* or *C*.

Sutherland states (p. 843): "Theory *C* yields incorrect predictions throughout with the exception of some of the initial findings". In only two cases (diamond and oblique rectangle; mirror images) do Sutherland's experimental findings tend to refute *C*, and in both cases the evidence is vitiated by failure to implement a simple and obvious control condition. In five cases under (8) and (12) *C* yields a correct prediction, where *A* yields a false one; so Sutherland's evaluation of his theory as superior to *C* can scarcely be upheld on the evidence of his own experiments. Moreover, one piece of his own evidence not quoted by Sutherland is the fact that, apparently, the octopus transfers from discrimination of filled (surface) shapes to outlined (contour) shapes[6]. Theory *A* is a theory of surface discrimination, not one of contour discrimination. For theory *A* a contour shape shares nothing in common with a congruent surface shape except its outline, and the theory must deal with that outline as a 'surface'. The resulting outputs will be very different from the outputs for a surface shape. This is illustrated in Fig. 3, which shows the theory *A* outputs for a square, (*a*) as a surface shape, and (*b*) as a contour shape. Quite evidently the two are very different, and no transfer should occur from one to the other.

Logical Weaknesses in Theory *A*

Sutherland gets around this difficulty by assuming ". . . that some sort of filling-in mechanism occurs with outline shapes, although no attempt will be made to specify how this takes place"[6]. This is scarcely an acceptable avenue of escape from what looks like a crucial objection to theory *A*. In making this assumption, Sutherland specifically eschews any interpretation of theory *A* as a theory of contour discrimination. While this removes some ambiguity from his original statement of the theory[2], it leads one to question its plausibility. There are good reasons, both logical and factual, for supposing that contour properties are more important in shape discrimination than surface properties. Logically this is so, because the property of 'shape' in a stimulus display entails the property of 'contour' in that display, but does not entail the property of 'surface' as this has been used in the literature, to describe a difference in brightness, hue or saturation between the interior and exterior of a closed contour. A discriminating system based on contour properties is thus more general than a system based on surface properties. Practically, it seems unlikely that shape discrimination could be based only on properties of surface extent and not contours, since it has been shown for at least two types of visual system that surface and contour have different central representations[7,8]. (Young's demonstration of a marked layering of the plexiform zone in the octopus[9] suggests that the various layers might mediate different aspects of a stimulus display, such as surface and contour). Although there is no direct evidence to show what the relations are between these two types of representation, the preceding argument about the greater generality of contour discrimination suggests that, in the absence of physiological or behavioural evidence that 'filling in' occurs, the proper strategy to pursue is to assume that discrimination of shape is based on contour properties. Experimenting with outlined shapes should then yield results uncontaminated by the possibility of competing or confounding computations based on surface extent (this was pointed out some time ago in assessing earlier work on visual discrimination of shape[4]).

A further weakness of theory *A*, which has been pointed out previously[10], is that the manner in which row and column outputs are analysed is vague. The statement of theory *A* (ref. 2) suggests that this further analysis involves not only the magnitudes of the outputs but also their spatial distributions. For example, theory *A* outputs for shapes *f* and *g* in Fig. 1 are identical except for the fact that the peaks in the horizontal outputs occur in different positions relative to the total extent of the horizontal outputs: to discriminate between the pair, the analyser must register this difference. That is, since the recognition of shapes is supposed to occur independently of their position in the two-dimensional array, the analyser must be capable of registering differences in spatial patterns independently of their position ('peak to the right', or 'peak to the left' must be registered independently of whether these occur to the left, to the right, or in the centre of the horizontal output, for example). This problem, of recognizing a spatial pattern independently of its position, has always been a major difficulty for neural theories about the recognition of shapes (a difficulty which is overcome, incidentally, in theory *C*, by transformation of spatial into temporal patterns). An analyser which would perform this function would be of considerable theoretical importance, but Sutherland's statements on the topic have been too nebulous to make it possible to decide how the analyser is supposed to work, or whether it can really cope with the problem of registering spatial patterns in different positions. Further, it is difficult to see what the point is of postulating a two-dimensional array to separate horizontal and vertical components of a shape before

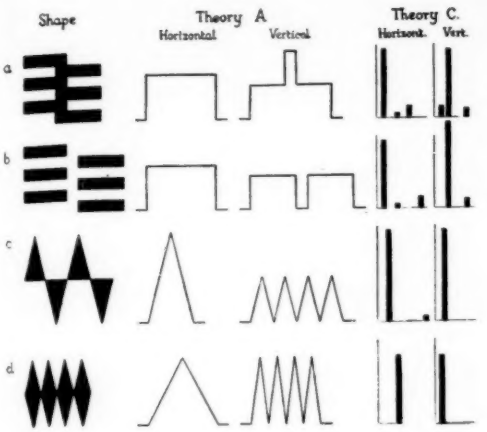

Fig. 4

input to the analyser. If the analyser has the properties required to deal with the analysis of the separate outputs of theory *A*, it should have the requisite properties for analysing shapes without prior separation into components, namely, the ability to register differences in spatial pattern independently of location.

Some New Evidence

In an attempt to gain some clear evidence in favour of either theory *A* or theory *C*, experiments were performed with rats and squirrels using the two pairs of shapes shown in Fig. 4. It can be seen by inspection of the associated outputs that theory *A* predicts good discriminability for the pair *a*, *b* and poor discriminability for the pair *c*, *d*: theory *C* predicts the reverse. These experiments will be reported in detail elsewhere; briefly, the results support theory *C* for both shapes in both rats and squirrels. In both cases there was a difference of discriminability in favour of the pair *c*, *d*. In the squirrel experiment the results are not quite conclusive, since only a small number of animals was available, and the experiment was in the nature of a pilot investigation. In the rat experiment, however, a *t*-test on the scores was significant ($P < 0{\cdot}01$), and in favour of theory *C*. It may be noted here that Sutherland has frequently reported significant differences for groups of animals in terms of a χ^2 test with one degree of freedom. Such a test is not appropriate as a test of differences between group means, since it takes no account of variability from one subject to the next. Thus, in addition to the difficulties already mentioned, a number of the results reported by Sutherland are based on inadequate statistical analysis; although the direction of the results is unambiguous, their conclusiveness is questionable.

The shapes used in the present experiment were surface shapes, rather than contour shapes. This may seem surprising, in view of the criticisms of the use of surface shapes made earlier. I used such shapes for the following reasons: (*a*) surface shapes should favour theory *A*, since that theory is a theory of surface discrimination, and it should involve complicating factors for a contour discrimination theory (namely, *C*); and (*b*) if (as seems scarcely possible) theory *A* could be interpreted as a contour discrimination theory, the use of surface shapes involves equal difficulties for both *A* and *C*. Thus, the use of surface shapes should either favour theory *A*, or be neutral between *A* and *C*: had contour shapes been used, the argument could have been put forward that the experiment was biased in favour of C.

Conclusion

To sum up: I believe that Sutherland's evaluation of theories *A* and *C* is faulty, and that the evidence he quotes supports theory *C* rather better than theory *A*. The faulty evaluation is in part due, apparently, to a misunderstanding of theory *C*. There are difficulties in his evidence for both theories, and either theory can only be saved by making additional assumptions.

Logical difficulties in theory *A* have been examined. Although the theory has not been stated clearly, it is evidently a theory which bases analysis on surface extent only, and not on the contours of figures. It seems unlikely that shape should be discriminated only in terms of surface and not in terms of contour, and physiological evidence to support this statement is available. This same evidence makes it perfectly plausible to suppose that contour can be 'abstracted' from surface shapes, and discrimination based on this abstracted information.

The analysis of horizontal and vertical outputs for theory *A* has been described quite as vaguely as the actual generation of those outputs, and to make the sort of analysis proposed comprehensible a far more thorough description is needed. Justification for separation into horizontal and vertical components is lacking, and this is a further weakness in the theory.

New evidence described here favours theory *C* over theory *A*, although the experimental conditions were biased in favour of *A*.

One may conclude by stating that neither on empirical nor on logical grounds is theory *A* superior to theory *C*. The logical weaknesses in theory *A* indicate that it should either be dropped or re-stated in a clear and unambiguous way.

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[1] Sutherland, N. S., *Nature*, **186**, 840 (1960).
[2] Sutherland, N. S., *Nature*, **179**, 11 (1957).
[3] Deutsch, J. A., *Nature*, **185**, 443 (1960).
[4] Dodwell, P. C., *Brit. J. Psychol.*, **48**, 221 (1957).
[5] Deutsch, J. A., *Nature*, **188**, 1090 (1960).
[6] Sutherland, N. S., *J. Comp. Physiol. Psychol.*, **53**, 95 (1960).
[7] Maturana, H. R., Lettvin, J. Y., McCulloch, W. S., and Pitts, W. H., *Science*, **130**, 1709 (1959).
[8] Gaze, R. H., *J. Physiol.*, **146**, 40 (1959).
[9] Young, J. Z., *Nature*, **186**, 836 (1960).
[10] Dodwell, P. C., *Nature*, **179**, 1088 (1957).

In the preceding communication Dodwell takes me to task for the way in which I interpreted[1] my own experimental results on shape discrimination by *Octopus*. I agree with some of his general remarks. For example, he emphasizes at the outset that no complete theory of shape recognition in any species has yet been proposed. All he or I have suggested are ways in which information from patterned visual

stimuli may be coded at some stage in the nervous system. These suggestions as to coding have been made to account for the relative difficulty animals have in discriminating between different pairs of shapes, the confusions they make between shapes, and the relative similarity of different shapes for a given species as revealed by transfer tests. A 'complete' theory would have to account for all the available evidence and would also be concerned not merely with how the stimuli are coded on the input side but exactly how the input as coded is further analysed and how the resulting neural output governs the responses made by the animal.

Unfortunately, I cannot agree with most of Dodwell's more detailed points. Many of them are based on assertion rather than argument. For example, he states that I use χ^2 to test for the difference between the performance of groups of animals, and rightly points out that this is the wrong statistic: in fact, in all papers written by me the significance of the difference between the performance of different groups of animals being trained to discriminate between different shapes has been evaluated by *t*-tests or analysis of variance and not by χ^2. Again, Dodwell suggests that the first stage of my own theoretical analysing mechanism (namely, breaking a shape up into its horizontal and vertical components) is unnecessary, since if the further analyser which analyses these projections "has the properties required to deal with the separate outputs of theory *A*, it should have the requisite properties for analysing shapes without prior separation into components": this is a complete *non-sequitur*. It is hard to see why Dodwell supposes that a mechanism which can analyse outputs on a one-dimensional neural array must be capable of analysing a two-dimensional array, and even if this were true there is no reason to suppose that the nervous system performs the complete analysis of shape in one stage.

Unfortunately, Dodwell does not state his own theory in the accompanying article. It may be helpful to the reader to have a brief statement of this theory[2]. He suggests that excitation representing the contours of shapes is projected on to a square array of cells in the brain. Impulses sweep across this square array in directions corresponding to the visual vertical and horizontal. Impulses travelling in either direction are said to be slowed down when they cross an area already excited by neural activity resulting from a contour present at the retina. The extent to which an impulse is slowed down is said to depend on the direction of the contour in relation to the direction in which the impulse is travelling. All impulses travelling in one direction are fed into a final common path: activity in this pathway represents the number of impulses arriving per unit of time. This theory is highly ingenious, but does not seem particularly plausible on neurological grounds, since it must involve some mechanism to synchronize the start of successive travelling waves of impulses. The pictures Dodwell presents in his Fig. 1 (column III) represent the outputs given by different shapes in the final common pathways: unfortunately, since he does not tell us how these outputs were derived, we have to take them on trust and many of them do not appear to conform to the rules he gives in his previous paper[2] (for example, those for shapes *b*, *c*, *j*, *l*, *n*, *o*). This doubt about how his outputs are derived makes it impossible to discuss some of the arguments based on these outputs. It may, however, be useful to discuss the crucial issues under each of Dodwell's main headings.

Evaluation in Relation to the Various Theories

Dodwell agrees that the ability of octopuses to discriminate between a diamond and an oblique rectangle constitutes a crucial test between his theory and my own. He is surely mistaken, however, when he says that the experiment I actually performed on this is inconclusive because I failed to equate the diamond and the rectangle for total brightness. He argues that since the area of the diamond used was 25 sq. cm. and the area of the rectangle was 20 sq. cm., it is possible that animals may have learned to discriminate between them in terms of the difference in the total brightness of each. This possibility is discussed in my paper reporting this experiment[3], and careful controls were run to test whether animals had been discriminating between the shapes in terms of their difference in brightness. Three findings reported in that paper rule out this interpretation: (1) If, after animals have learned the discrimination they are shown a rectangle with a *larger* area than the diamond, then, if they are responding in terms of brightness, they should respond to it in the way in which they had learned to respond to the diamond. When a large rectangle was shown to animals, they continued to respond to it in the way in which they had formerly responded to the original rectangle. (2) Similarly, when a diamond of *smaller* area than the original rectangle was shown, they continued to respond to it as they had done to the original diamond. (3) Animals were originally trained with white figures shown against a grey background: they continued to discriminate between a diamond and an oblique rectangle when they were presented with black shapes against the same background. The brightness of the black diamond was less than that of the original white rectangle. Despite these findings, Dodwell writes, "no direct test of the effect of the brightness difference was made".

Of the remaining points raised by Dodwell in this section, he is correct in saying that his theory (as well as mine) predicts transfer from a vertical rectangle to a broken vertical rectangle (*a*, in Fig. 1), and I was mistaken in supposing that it could not[1]. His other arguments, however, are vitiated by a failure to take into account the results of transfer tests. Thus, when octopuses are trained to discriminate between shapes which differ markedly in amount of contour (for example, shapes *j*–*q*), the ways in which they effect transfer show that something more is involved than an analysis along the horizontal and vertical axes. This means that no theory based merely on an analysis along these axes will account for all the results, and hence it is necessary to postulate some further analysing mechanism at work.

Logical Weakness in Theory A

Much of this section is taken up with a general accusation of vagueness in my own theory. As pointed out already, this charge is justified but applies to all theories of shape recognition hitherto proposed. However, it should be remembered that my own theory has been able to yield many detailed predictions which could be and have been tested, and that these predictions were rigorously derived from the theory Dodwell does not deny. As Dodwell rightly observes, the greatest area of vagueness lies in the

fact that further mechanisms for analysing the output from rows and columns (that is, the shape of the 'projections' shown in Fig. 1 of Dodwell's article) have not been specified (for some suggestions see ref. 4). It would, however, be idle to speculate about this problem in default of evidence which would allow one to choose between alternative possibilities, and until the theory has received more thorough testing at its present level of complexity. Dodwell himself has not attempted to specify how the outputs yielded by his own mechanism are analysed.

He raises the important question of whether shapes are analysed in terms of their surface area or whether they are analysed in terms of their contours, and writes that my suggestion of a filling-in mechanism is vague. It is of course true that the contours of a shape are of importance in shape recognition, but what I am arguing is that it is the distances between contours that is important, not merely their orientation. Dodwell's mechanism operates primarily on the direction of contours in visual space: it does not analyse the distances between contours. It is for this reason that it predicts that animals should be unable to discriminate between a diamond and an oblique rectangle. All the contours of these figures run at 45° to the vertical and it is possible to draw the figures with the same total amount of contour as one another. It seems certain that, in discriminating between shapes, animals take account of the separation between contours and not merely of the directions in which they run, and for this reason Dodwell's theory is implausible. According to my theory, the octopus computes horizontal and vertical distances between contours: this would involve a filling-in mechanism for outline figures (incidentally, transfer to outline figures by octopuses is poor and sometimes non-existent[3,5]). It is important to be clear that a mechanism which operates *solely* on contour does not avoid the difficulties inherent in a filling-in process: thus if all contours present in a shape are analysed, animals that have learned to discriminate between unfilled outline figures should not effect transfer to the same figures with further contours inscribed within them. Evidence on this is lacking for the octopus, but Lashley[6] has found that rats show excellent transfer in just these circumstances. Thus a theory such as Dodwell's would need a 'blotting-out' mechanism to enable animals to disregard internal contours: this would be as complex as a 'filling-in' mechanism and indeed logically the two mechanisms reduce to the same thing.

Some New Evidence

In his final section Dodwell cites an interesting experiment he performed on rats and squirrels as providing evidence in favour of his own theory and against mine. He asserts that of the shapes shown in his Fig. 4, my theory (*A*) must predict that *a* and *b* would be more readily discriminable than *c* and *d*, whereas his own predicts greater discriminability of *c* and *d* than of *a* and *b*. These predictions are made on the basis of the outputs yielded according to the two theories and these outputs are shown in Fig. 4. The prediction Dodwell makes hinges on the difference between the outputs of *a* and *b* under my theory (*A*) being greater than the difference between the outputs of *c* and *d*, and vice versa under his theory (*C*). Examining the outputs in question as shown in Fig. 4, it is quite impossible to say under either theory whether the outputs of *a* and *b* are more or less different from one another than those of *c* and *d*: the reader can verify this for himself by inspection of this figure. Hence Dodwell's results, although of interest in their own right, are inconclusive with respect to the two theories under discussion. In order to say whether the outputs are more different for one pair of shapes than for another, it would be necessary to specify how the outputs themselves are analysed, which—as Dodwell himself points out—has not been done. Both theories can be made to make predictions, but only where the output for one pair of shapes is the same and the output for another different: Dodwell makes this point at the start of his section on evaluation, but he appears to have lost sight of it in designing a test between the two theories.

Conclusion

It is perhaps worth emphasizing why I have made no attempt in the above to deal individually with all the points raised by Dodwell: the details of this controversy are in fact in danger of obscuring the value of the experimental work on which the controversy is based. Dodwell and I are in agreement about the methods and aims of this type of work, and he clearly agrees that the evidence demonstrates that in the octopus an analysis of shape along horizontal and vertical co-ordinates is occurring. His own theory is valuable as a research tool, and this is all that I have claimed for my own. His theory prompted me to undertake the experiment on diamonds and oblique rectangles mentioned above, and this experiment yielded results of considerable interest irrespective of the rights and wrongs of any particular theory. The real importance of this type of experimental work lies in two aspects: (1) It demonstrates that it is possible to put forward hypothetical neurological mechanisms to explain behavioural findings, and that these mechanisms can be tested at a behavioural level. Disagreement arises at the present moment because none of the theories proposed has received sufficient systematic testing, and also none of the theories is complete. (2) Perhaps more important, the findings that have been obtained must eventually be linked with findings on the neuroanatomy and neurophysiology of the optic pathways. Only by having data on the functions mediated by these pathways will it be possible to interpret the meaning of the neuroanatomical structures and neurophysiological relationships which are at present being uncovered (cf. Young[7]). The final verification of any theory of shape discrimination must wait on further knowledge of this sort.

The work discussed in this article forms part of a project on "Stimulus Analysing Mechanisms", which is financed jointly by the American Office of Naval Research (Contract *N* 62558–2453) and the Nuffield Foundation. I am grateful to these bodies for their financial support.

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[1] Sutherland, N. S., *Nature*, **186**, 840 (1960).
[2] Dodwell, P. C., *Brit. J. Psychol.*, **48**, 221 (1957).
[3] Sutherland, N. S., *J. Comp. Physiol. Psychol.*, **53**, 95 (1960).
[4] Sutherland, N. S., *Nature*, **179**, 1310 (1957).
[5] Sutherland, N. S., *J. Comp. Physiol. Psychol.*, **53**, 104 (1960).
[6] Lashley, K. S., *J. Gen. Psychol.*, **18**, 123 (1930).
[7] Young, J. Z., *Nature*, **186**, 836 (1960).

LETTERS TO THE EDITORS

GEOPHYSICS

Connexion between Micropulsations and the Ionosphere

Duffus has recently pointed out a connexion between P_c occurrence and the F-region[1]. Other workers[2] have found correlation between P_t and f_0F_2 and it can reasonably be asserted that the ionosphere is a major factor in the transmission path by which these characteristic geomagnetic micropulsations are observed at ground-level. Recent work here has offered some further evidence on this subject.

For the purpose of this investigation P_c are defined as pulsations with a considerable element of continuity, lasting a number of hours, with periods between 10 and 60 sec., usually with a characteristic 'beating' appearance, and occurring predominantly in the day-time, although this last factor is not used for identification. P_t are short trains of oscillations with periods around 60 sec. which occur usually in otherwise quiet times at night. Fig. 1 shows typical examples of each class recorded on the Signals Research and Development Establishment's magnetometer. While it is generally accepted that these oscillations have their primary origin in solar emission, which by interaction with the Earth's atmosphere and magnetic field excites resonances in several possible ways[3–5], the exact manner of formation of each kind of pulsation is still unknown.

As part of a wider geomagnetic investigation, recording magnetometers have been set up at Christchurch (51° N., 2° W.) and Ascension Island (8° S., 14·5° W.), approximately 6,750 km. apart. Ascension is close to the geomagnetic equator so there can be no common lines of force with Christchurch. Inspection of simultaneous recordings from the two stations shows that P_t occur on both traces at the same time and with the same period, within the limits of discrimination of the system (Fig. 2). The amplitude at Christchurch is some 20–30 times that at Ascension. On the other hand, P_c, although occurring at about the same time and over the same frequency range, do not exhibit the same coherence (Fig. 3).

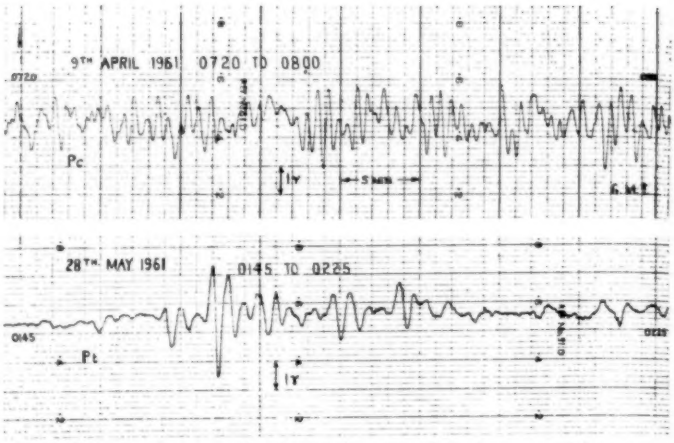

Fig. 1. Examples of geomagnetic micropulsations P_c and P_t observed at Christchurch, Hants

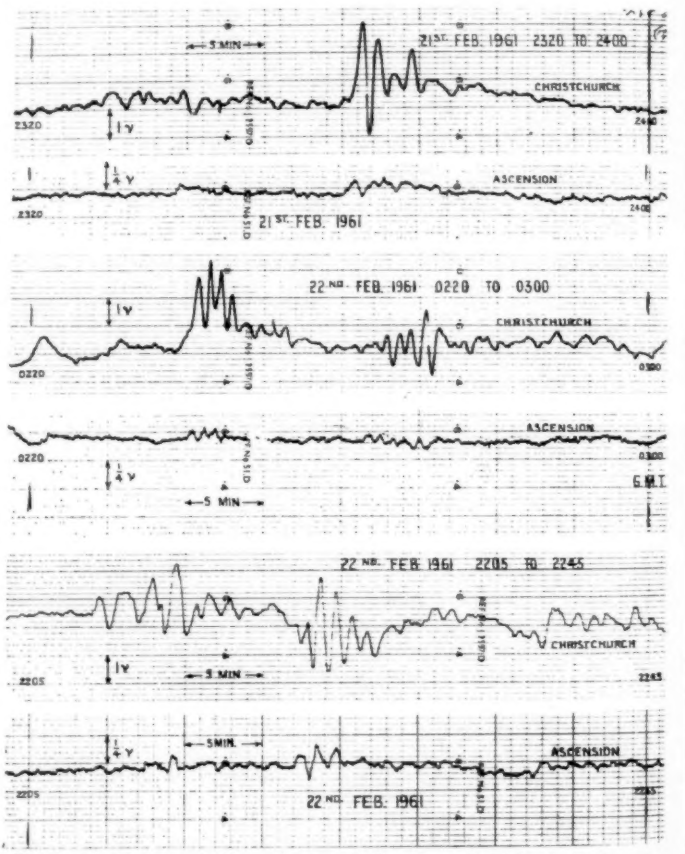

Fig. 2. Simultaneous recordings of micropulsations P_t at Christchurch, Hants, and Ascension Island

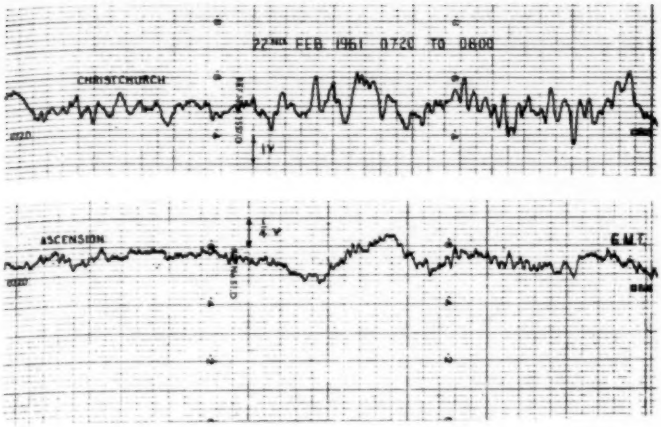

Fig. 3. Simultaneous recordings of micropulsations P_c at Christchurch, Hants, and Ascension Island

The seasonal variation of the start-up time (here defined as the time of the first fifth of the day's total active time) of P_c observed at Christchurch shows a definite correlation with sunrise (Fig. 4). This confirms the conclusion of Dr. Duffus that the low atmosphere has a modulating control over P_c and it also shows that this control varies markedly with latitude, in contrast to P_t.

The problem is to explain why P_t are night-time phenomena and are observed to be coherent over a great latitude-range, being of greater amplitude at high latitude, while P_c are recorded in the day-time and vary with latitude, both classes of pulsation being linked with the ionosphere. P_t may be oscillations of the outer atmosphere which are coupled into the ionospheric duct at the auroral belt, being propagated only in the low-attenuation night-time state of the ionosphere, while P_c may have a much more local and resonant transmission system, still originating in an outer atmosphere oscillatory mode. It is possible that part of their oscillatory content is inherent in the arriving corpuscular stream. Granulation waves of this frequency are known to exist in the photosphere[6–8].

Work at this Establishment is proceeding on the examination and classification of micropulsation activity together with a search for periodicities and cross-relationships. It is proposed to set up a magnetometer system with accurate timing between the stations and an increasing frequency coverage.

Acknowledgment is gratefully made to Mr. L. A. Brackstone of the Department of Scientific and Industrial Research at Ascension for his helpful co-operation.

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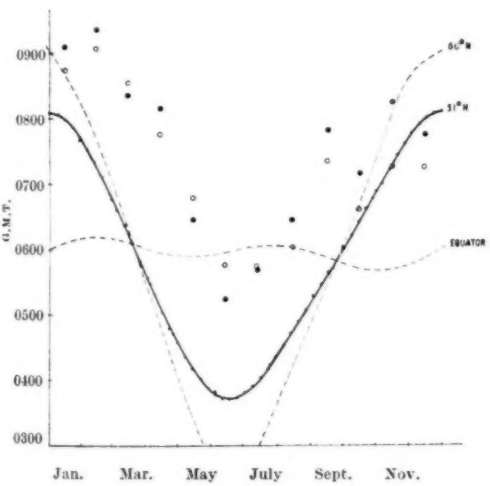

Fig. 4. Relation between mean monthly P_c start-up time and sunrise time at Christchurch, Hants. (Certain P_c are clear unambiguous displays. Weighed P_c have the hours of marginal P_c occurrence divided by 2 and added to the certain P_c.) Full line, sunrise 51° N.; ○, time of 1/5 days score weighed P_c; ●, time of 1/5 days score certain P_c; – – –, sunrise 60° N. and equator

[1] Duffus, H. J., *Nature*, **188**, 719 (1960).
[2] Afanasieva, *Proc. I.U.G.G. Meeting*, Utrecht (Sept. 1959).
[3] Dungey, J. W., *Ionospheric Res. Lab., Pennsylvania State Univ., Sci. Rep.*, No. 69 (Sept. 15, 1954).
[4] Kato, Y., and Akasofu, S., *Sci. Rep. Tohoku Univ.*, Ser. 5, *Geophys.*, **7**, No. 3 (March 1956).
[5] Obayashi, T., and Jacobs, J. A., *Geophys. J. Roy. Astro. Soc.*, **1**, No. 1 (1958).
[6] Alfven, *Cosmical Electrodynamics*, 147.
[7] Piddington, J. H., *Mon. Not. Roy. Astro. Soc.*, **114**, 638 (1954).
[8] Piddington, J. H., *Mon. Not. Roy. Astro. Soc.*, **116**, 314 (1956).

Stress Effects on Thermoremanent Magnetization

Hall and Neale[1] have reported accurate measurements of the directions of thermoremanent magnetization acquired by rock specimens which cooled under stress in a low magnetic field. They observed small, but consistent, differences between the directions of thermoremanent magnetization in rocks cooled under stress and without stress, and suggested that their observations conflicted with our results[2,3], which indicated the absence of measurable differences in isotropic rocks. However, we consider that the experiments of Hall and Neale are consistent both with our results and with our conclusions, which may be summarized as follows.

In an igneous rock which is cooled under stress in a small magnetic field and then released from the stress, the direction of thermoremanent magnetization differs from the field direction by an angle which is controlled both by the stress and by the intrinsic anisotropy of the rock, but if the intrinsic anisotropy is very small, then the angular difference is also very small, regardless of the stress which was applied[4] (at least up to 1,000 kgm./cm.²).

In our experiments, rocks which were shown by torque-meter experiments[5] to have anisotropies less than about 2 per cent were cooled under directed stresses up to 1,000 kgm./cm.[2] in the Earth's field. Within our measurement error of 2°–3° we found that the thermoremanent magnetization after release of stress coincided with the field direction. Quite different results were obtained with anisotropic rocks; for example, in a rock with approximately 60 per cent anisotropy which was cooled under stress, thermoremanent magnetization was induced at angles between 5° and 60° away from the field direction. It is important to note, therefore, that although Hall and Neale have reported more accurate measurements of the directions of such magnetization in rock specimens, they were using specimens with appreciable intrinsic anisotropies, such that the magnetization acquired in the absence of stress differed from the field direction by as much as 4°. According to Stacey's[3] analysis, this implies intrinsic anisotropy of at least 13 per cent, which can scarcely be described as 'slight'; a limit of 10 per cent was suggested[5] as acceptable in rocks used for palæomagnetism.

Further experiments are needed on the interaction of stress and the intrinsic magnetic anisotropies of rocks. It may be necessary to impose a more stringent limit than hitherto on the anisotropy, which is acceptable in rocks used for palæomagnetism.

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[1] Hall, J. M., and Neale, R. N., *Nature*, **188**, 805 (1960).
[2] Stott, P. M., and Stacey, F. D., *Nature*, **183**, 384 (1959).
[3] Stott, P. M., and Stacey, F. D., *J. Geophys. Res.*, **65**, 2419 (1960).
[4] Stacey, F. D., *Nature*, **188**, 134 (1960).
[5] Stacey, F. D., *J. Geophys. Res.*, **65**, 2429 (1960).

From the results of our pressure experiments[1] and more recent measurements of susceptibility anisotropy it appears that this parameter is of negligible importance in the production of thermoremanent pressure effects.

The scatter of thermoremanent magnetization directions mentioned in our previous communication is a maximum for the coarse-grained Ob Lusa dolerite rock type. A group of ten finer-grained contact specimens from the Ob Lusa dolerite dyke show a scatter of only $\pm 1{\cdot}5°$ about the mean direction (the ambient field direction), while showing a pressure-induced rotation of moment six times larger than that of the coarser specimens. It is these latter specimens that show a maximum scatter of $\pm 4°$ about the ambient field direction.

Measurements of susceptibility anisotropy at low fields have been made at Cambridge on all the specimens used in the investigation. Anisotropies of 1 per cent or less between axial values of initial susceptibility were found for all the rock types employed with the exception of that having pyrrhotite as a magnetic component.

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[1] Hall, J. M., and Neale, R. N., *Nature*, **183**, 805 (1960).

ASTRONOMY

Holistic Approach to Selenology

The proximity of exploration of the surface of the Moon demands a critical review of present selenological hypotheses. I wish to point out a defect inherent in modern selenological thought and to stress that there is a need for more information before developing contemporary theories more fully.

Theories of the formation of lunar craters may be divided into two classes: extrinsic (that is, impact—favoured by Baldwin and Urey) and intrinsic (that is, igneous—championed by Jeffreys and Spurr). Broadly speaking, the extrinsic hypotheses appeal to macroscopic features (for example, predominant circularity, the depth-diameter relationship, and the near-random distribution of craters) while the intrinsic theories appeal more to the finer features (including crater chains, summit craterlets, and crater polygonality).

The limitation to both approaches is that neither (in the first instance) distinguishes between primary and secondary features of the lunar surface.

Primary features are the large-scale structures as seen immediately after they were formed, and by secondary features I mean the alterations to the primary structures during their later life (this includes the effects of stress systems and erosion).

Consideration of the lunar grid system shows that large-scale stresses have been applied to the lunar surface during its history, and the effects of these stresses have been to obliterate information pertinent to the origin of the craters. In addition, completely new types of feature have arisen (for example, rill and valley systems) and some of these have been erroneously interpreted as primary features. Thus when one accepts the fact that the valleys radial to the Mare Imbrium are nothing more than a slightly more prominent element of the general grid system the collision hypothesis for the origin of this Mare loses considerable weight.

I would suggest, therefore, that the need at the present time is for a more expiscate approach. Many details of the lunar surface found recently do not fit in comfortably with any existing theories of crater formation, nor indeed with present ideas on secondary activity. We can only attempt to construct a complete theory when we have realized more of the properties of the features with which we are dealing.

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Fielder, G., *Structure of the Moon's Surface* (Pergamon Press, London, 1961).
Warner, B., *J. Brit. Astron. Assoc.*, **71**, 116 (1961).
Warner, B., *J. Brit. Astron. Assoc.* (to be published).

PHYSICS

Use of a Different Type of Fluorescent Material as an Optical Maser

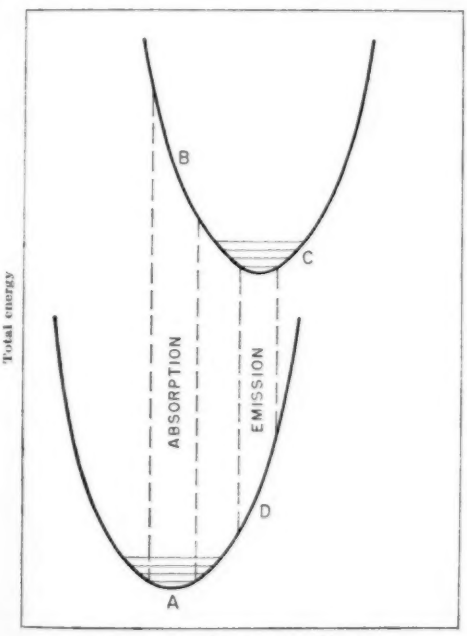
The recent interest in the development of infra-red and optical maser systems has involved a number of different basic principles[1–3]. This communication is concerned with an alternative concept which utilizes the Stokes's shift associated with absorption and emission bands created by point defects in solids.

Preliminary calculations indicate that such a device can be made to operate.

We will be concerned with defect centres which possess non-overlapping absorption and emission bands. A specific example is the *F*-centre in potassium chloride, and this centre is selected as a representative example on which detailed calculations are performed since many of its properties are known. It is believed to consist of an electron trapped at a negative-ion vacancy. The broad absorption band associated with this defect centre shows a peak at 5600 Å. (2·3 eV.) while the emission band lies at 1·0μ. At temperatures below 80° K., the absorption-emission process is very nearly 100 per cent efficient so that for each photon absorbed one is emitted[4].

One can describe the principles involved in this suggested device by introducing a configuration co-ordinate curve (Fig. 1). The total energy is plotted along the ordinate, while the abscissa represents a general configuration co-ordinate. The frequency shift which occurs between the absorption and emission bands can be explained by referring to Fig. 1. The system in its ground-state at A undergoes the transition $A \rightarrow B$ when it is stimulated by the appropriate radiation. Then a radiationless transition, $B \rightarrow C$, follows, which lowers the vibrational level of the excited state. The system decays to a high vibrational level of the ground-state through the radiative transition, $C \rightarrow D$. Finally, the system relaxes back to A. Thus, the entire process may be visualized as a series of transitions between levels A, B, C and D. In view of the experimental data on emission, one may be certain that thermal equilibrium is established in the upper state before the emission of a photon. The non-radiative transitions from C to D are known to be small.

The relaxation times for the phonon-emitting transitions, $B \rightarrow C$ and $D \rightarrow A$, are approximately 10^{-12} sec., while the photon-emitting transition takes about 10^{-8} sec. Thus, it should be possible to make the ratio of populations in states C and D large by injecting radiation of the frequency $\nu_a = (E_b - E_a)/h$. On exposure to radiation of a second frequency, $\nu_e = (E_c - E_d)/h$, downward transitions are induced, resulting in the amplification of this second signal.



Generalized configuration co-ordinate

Fig. 1

The degree of amplification attained from such a system can be studied in terms of several parameters. The intensity of radiation emitted, I, is given by the relationship:

$$I = I_0 e^{\alpha' x} \qquad (1)$$

where I_0 is the stimulating signal incident on the crystal, x is the distance the light travels and α' is a constant which corresponds to the absorption co-efficient in the case of absorption. The absorption coefficient of a crystalline material containing defect centres is given by Smakula's equation as[5]:

$$\alpha = \frac{2}{9}\frac{e^2 f}{mc}\frac{(n^2+2)^2}{n^2}\frac{1}{\Delta\nu}N_a \qquad (2)$$

where $\Delta\nu$ is the absorption band-width at half intensity; f is the oscillator strength associated with the defect centre; n, is the refractive index of the host crystal; N_a is the concentration of centres in the ground-state over that in the excited state; and e, m and c have their usual meanings.

If one now replaces $\Delta\nu$ with $\Delta\nu'$, the width at half height of the emission band, and N_a by N_c, one can adopt equation (2) to the case of emission since for any temperature of interest, $N_c \gg N_a$. In the case of the *F*-centre in potassium chloride:

$$\alpha' = 2{\cdot}7 \times 10^{-16} N_c \qquad (3)$$

where f has been set equal to 0·81. If $N_c = 10^{14}$, we find from equation (1) that x must be of the order of meters if one is to achieve a substantial gain; however, a measurable amplification could be attained if x were of the order of cm.

Consider next the power requirements to attain 10^{14} centres in state C. The life-time of this state in the absence of stimulating radiation is given by the einstein A coefficient as $5{\cdot}5 \times 10^7$ sec. when the effects of the dielectric are neglected. Hence, the life-time of the state C is $1{\cdot}8 \times 10^{-8}$ sec. and the required pumping power to maintain 10^{14} centres in state C is:

$$P = \frac{(h\nu)10^{14}}{1{\cdot}8 \times 10^{-8}} = 1{\cdot}27 \times 10^{22}\ \text{eV./sec.} = 2{,}000\ \text{W.}$$

This is excessive if one hopes to maintain the required temperature; however, one may reduce this requirement by varying several parameters. The crystal dimensions could be reduced to 1 cm. × 1 cm. × 0·1 cm., thus reducing the power by a factor of 10. Furthermore, the device may be operated intermittently. Hence, the pumping power is reasonable for experimental purposes.

It should be pointed out that heavy *F*-centre concentrations will introduce non-linear effects owing to greater absorption at the face of the crystal than in its interior; however, these effects should not seriously affect the performance of such a device.

One mode of operating such a device would involve a thin crystal, two sides of which would be silvered and approximately parallel. The silvered sides would have two small apertures, thus permitting a

long path for the radiation which is to be amplified due to multiple reflexions. The pumping radiation would fall on the face of the crystal at right angles to the stimulating radiation and would traverse the crystal in its shortest dimensions.

The noise inherent in such a system would be the result of spontaneous emission within the crystal: however, this spontaneously emitted radiation would be contained in a 4π steradian solid angle and very little of it would be travelling in such a direction as to emerge from the crystal through the small exit aperture. One may most probably measure the spontaneous emission emerging from a small exit aperture. However, we believe that the stimulated emission will be much greater under operating condition and the noise should not be a major problem. Thus, the spontaneously emitted radiation will lower the density of systems in the excited state, but should not be a serious limitation from the standpoint of noise.

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[1] Maiman, T. H., *Nature*, **187**, 493 (1960); *Brit. Comm. and Electron.*, **7**, 674 (1960).
[2] Sorokin, P. P., and Stevenson, M. J., *Phys. Rev. Letters*, **5** (7), 557 (1960).
[3] Javan, A., Bennett, jun., W. R., and Herriott, D. R., *Phys. Rev. Letters*, **6** (3), 106 (1961).
[4] van Doorn, C. Z., and Haven, Y., *Philips Res. Rep.*, **11**, 479 (1956). Becker, K., and Pick, H., *Nachr. Akad. Wiss. Gottingen*, Math.-physik. Kl., **2**, *a*, 167 (1956).
[5] Markham, J. J., *J. Phys. Chem.*, **57**, 762 (1953).

Dimensions of Electrical and Magnetic Quantities

FOR rather more than ten years the rationalized M.K.S. system of units has been generally accepted as the most satisfactory one to employ for practical calculations involving electrical and magnetic quantities[1,2]. This system side-steps the question of dimensions by introducing two unfortunate expressions—permittivity and permeability of free space—and assigning to them, respectively, the values $10^{-9}/36\pi$ farad/metre and $4\pi \times 10^{-7}$ henry/metre. Any dislike aroused by these terms may, however, be easily overcome by giving them new names: electric factor and magnetic factor, the question of units being left until later.

Unfortunately, the situation regarding dimensions is less satisfactory and still remains unsettled. L. H. A. Carr[3] has invoked the special theory of relativity to show that dimensionally Coulomb's law for the force F between charges q_1 and q_2 at a distance r apart in a uniform medium is $[F] = [q^2\ L^{-2}]$, and from this relation all electrical and magnetic quantities can be expressed solely in the dimensions mass, length and time. Carr's proposal, however, does not seem to have been taken up and is not even mentioned in recent text-books[4,5].

An alternative suggestion is to regard q as a dimension in its own right[2]; but this settles nothing and leads to the singularly useless result that electromotive force $[E] = [ML^2\ T^{-2}\ q^{-1}]$, implying that E is inversely proportional to q, whereas in fact the inverse is true. It is the purpose of this communication to advance evidence in support of Carr's proposal and to demonstrate that it leads to a very satisfactory direct physical relationship between electrical and magnetic quantities and their dimensions.

The gravitational force of attraction F between two masses is given by $[F] = [G\ M^2\ L^{-2}]$, where G is the so-called gravitational constant and is necessary to make the equation balance dimensionally. However gravitational attraction is independent of the medium and the introduction of G is quite arbitrary. Mass is only a convenient way of assessing the quantity of matter in a body, and it happens that in the gravitational equation its use leads to a rather awkward result dimensionally. An equally legitimate method for assessing a quantity of matter is to count protons or electrons, a neutron being supposed for this purpose to contribute one of each. It follows then that if two bodies are represented by $N_1\ q_0$ and $N_2\ q_0$, where q_0 is the elementary charge and N_1 and N_2 are pure numbers, $[F] = [q^2\ L^{-2}]$ when $[M]$ in the gravitational equation is replaced by $[q]$ and $[G]$ is discarded as being unnecessary. From this it follows that $[q] = [M^{\frac{1}{2}}\ L^{\frac{3}{2}}\ T^{-1}]$, in agreement with Carr. It has been objected that dimensions with fractional exponents are meaningless, but this is merely a point of view, for if $[q]$ is taken as the fundamental quantity rather than $[M]$, then $[M] = [q^2\ L^{-3}\ T^2]$ and the fractions disappear. Even though the relation $[q] = [M^{\frac{1}{2}}\ L^{\frac{3}{2}}\ T^{-1}]$ is not very attractive, the expression for the energy of a charged sphere, $\frac{1}{2}q^2/r$, is simple enough and the dimensions of q follow directly from this. Having established the dimensions of charge, it follows that $[E] = [M^{\frac{1}{2}}\ L^{\frac{1}{2}}\ T^{-1}]$. This relationship is equivalent to $[E] = [F]^{\frac{1}{2}}$ and is quite satisfactory since the force of attraction between two similar parallel plates, oppositely charged, can be directly measured and is found experimentally to be proportional to E^2, where E is the electromotive force between them.

The energy stored in a capacitor is $\frac{1}{2}CE^2$ and it follows therefore that $[C] = [L]$, and since E^2/R is the rate of doing work, $[R] = [L^{-1}\ T]$. Combining $[R]$ and $[C]$ we have $[R.C] = [T]$ and this result is independent of the nature of the medium. The identification dimensionally of RC with time is not difficult to accept when it is realized that the important criterion is that it shall be possible to measure time by means of a combination of resistance and capacitance. This is done by charging the capacitor to a convenient voltage E and connecting a resistor across its terminals. After time RC the voltage has dropped to $1/e$ of its original value, where $e = 2{\cdot}718$.

It is sometimes argued that capacitance cannot be physically equivalent to length, even though the capacitance of a sphere is proportional to its radius; but employing the criterion used for RC, namely that the dimension in question must be measurable by the quantity or quantities concerned, all that is necessary is to charge a parallel plate capacitor to a given voltage and then observe the rise in voltage as the plates are separated. The voltage is proportional to the distance apart of the plates and therefore the voltmeter used can be directly scaled to indicate distance.

Resistance also may be used to measure length in a direct manner since the resistance of a wire of constant cross-section is proportional to its length. However, resistance varies as length divided by area of cross-section, that is, $[L^{-1}]$, and in fact it is proportional to $[L^{-1}\ T]$, which is reasonable since for a fixed applied voltage resistance is inversely proportional to the mean velocity of electron flow. The impedance of free space (nearly 377 ohms) is obtained by multiplying the velocity of light in m./sec. by the electric factor $10^{-9}/36\pi$ and taking the reciprocal. Dimen-

sionally this checks with $[R] = [L^{-1}\ T]$, for now, of course, the electric factor is dimensionless. The law of force between two charges in a uniform medium becomes $F = q_1\ q_2/\alpha\ r^2$, where α is proportional to the dielectric constant of the medium and is a pure numeric.

The dimensions of magnetic pole, m, are readily obtained from the relation $[m.q] = [M\ L^2\ T^{-1}]$ which is independent of the medium. From this equation, $[m] = [M^{\frac{1}{2}}\ L^{\frac{1}{2}}]$ and the law of force between two magnetic poles, m_1 and m_2, spaced a distance r apart in a uniform medium is given by $F = m_1\ m_2\ c^2/\beta r^2$, where c is the velocity of electromagnetic radiations *in vacuo* while β is proportional to the permeability of the medium and is a pure numeric. The relation $[m] = [M^{\frac{1}{2}}\ L^{\frac{1}{2}}]$ may not look promising but for a U-shaped magnet with poles at its extremities, the force between the poles produces a bending moment in the magnet and a very direct connexion between $[m^2]$ and $[ML]$ is apparent. The magnetic factor applicable to the rationalized M.K.S. system is proportional to β/c^2 and should therefore be given in units of sec.^{2}/m.^{2}.

The equation $[F] = [q^2\ L^{-2}]$ is the only one which leads to the plausible relationships discussed above. More general equations are $F = \dfrac{q_1\ q_2}{\alpha\ \varkappa_0\ r^2}$ and $F = \dfrac{m_1\ m_2}{\beta\ \mu_0\ r^2}$, where $\varkappa_0$ and μ_0 represent unknown properties of free space and $\varkappa_0\mu_0 = 1/c^2$. Let $\mu_0 = 1$ and $\varkappa_0 = 1/c^2$, from which we obtain $[E] = [M^{\frac{1}{2}}\ L^{\frac{1}{2}}\ T^{-2}]$, $[C] = [L^{-1}\ T^2]$ and $[R] = [LT^{-1}]$. These expressions cannot be simply related to the nature of the quantities concerned and, for example, capacitance becomes the inverse of acceleration, while resistance has the dimensions of velocity, the inverse of what might reasonably be expected.

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[1] Schelkunoff, S. A., *Proc. Inst. Radio Eng.*, **36**, 827 (1948).
[2] Symposium, *Proc. Inst. Elec. Eng.*, Part 1, **97**, 235 (1950).
[3] Carr, L. H. A., *ibid.*
[4] Bleaney, B. I., and Bleaney, B., *Electricity and Magnetism* (Clarendon Press, 1957).
[5] Smith, C. J., *Electricity and Magnetism* (Edward Arnold (Publishers), Ltd., 1959).

CHEMICAL ENGINEERING

Inversion of a Laplace Transform arising from a Problem in Applied Chemical Kinetics

The solution to a problem (Wood, T., unpublished work) arising from an analysis of a closed circuit consisting of a stirred-tank reactor and a tubular reactor in series, with first-order, isothermal, irreversible kinetics, involves finding the inverse of the Laplace transform :

$$\bar{c}(s) = \frac{1}{s + \beta_1 - \beta_2 . \exp(-\beta_3 s)}$$

where β_1, β_2, β_3 are known, positive, constants. The transform can be re-arranged:

$$\bar{c}(s) = \frac{\dfrac{1}{(s + \beta_1)}}{1 - \dfrac{\beta_2}{(s + \beta_1)} . \exp(-\beta_3 s)}$$

Since no tabulated transform could be found, the denominator was expanded as a power series, giving:

$$\bar{c}(s) = \sum_{n=0}^{\infty} \left[\frac{\beta_2{}^n}{(s + \beta_1)^{n+1}} . \exp(-n\beta_3 s) \right]$$

The inverse of the transform is now readily shown to be[1]:

$$c(t) = \sum_{n=0}^{\infty} \frac{\beta_2{}^n}{n!} . (t - n\beta_3)^n . \exp\{-\beta_1(t - n\beta_3)\} . H(t - n\beta_3)$$

where $H(t - n\beta_3) = 0$ when $t \leqslant n\beta_3$; $H(t - n\beta_3) = 1$ when $t > n\beta_3$.

The validity of the solution in this particular case was proved by its ability to satisfy the original problem and the given initial and boundary conditions. It would be of interest to know, in the general case, the conditions for which the approach adopted above is valid.

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[1] Carslaw, H. S., and Jaeger, J. C., *Operational Methods in Applied Mathematics* (Oxford Univ. Press, 1953).

GEOLOGY

Continuation of the Great Glen Fault beyond the Moray Firth

The Great Glen Fault was shown by W. Q. Kennedy[1] to be a sinistral tear fault on which a displacement of about 65 miles has occurred since Middle Old Red Sandstone time. E. M. Anderson[1] considered that "the curvature of the fault trace may indicate that we are dealing with the south-western sector of a major fault and that the main extension of the dislocation will probably lie to the north-east". This curvature is a gradual change of strike from about 040° in Loch Linnhe to about 035° at Inverness.

Examination of the submarine topography of the Moray Firth, Orkney and Shetland areas shows that a possible course for the Great Glen Fault lies through Shetland to the edge of the continental shelf north of Shetland[2]. The features which seem to indicate the continuation of the fault in this direction are shown in Fig. 1. Between Smiths Bank and the Caithness coast, a shallow submarine valley is especially well shown by the 30-fathom contour; the axis of this valley has a strike of 035° like the Great Glen Fault at Inverness. However, to pass along the axis of the valley, the strike of the fault north-east of Inverness has to change from 035° to 040° and then back again to 035° over a distance of about 40 miles.

To the north, the sea-floor east of Orkney is rather flat and featureless, and provides no evidence. If the fault crosses it with a gradual swing in strike towards the north, it runs into, and along, a trench to the south-west of Fair Isle shown up by the 50-fathom contour. The axis of this trench has a strike of about 025° to the south-west of Fair Isle and swings round to about 020° in its continuation up the west side of Shetland; it reaches its greatest depth of more than 70 fathoms at its northern end in a latitude of 60° N.

At the northern end of the trench, if the fault passes just east of the deepest part and to the west of The Deeps (a narrow submarine valley extending beyond the end of the trench and shown up by the 50-fathom contour) it runs ashore in Shetland at the head of Seli Voe and joins on to the Walls Boundary Fault[3]. The Walls Boundary Fault can be followed overland until it runs into the sea at Aith Voe. Still

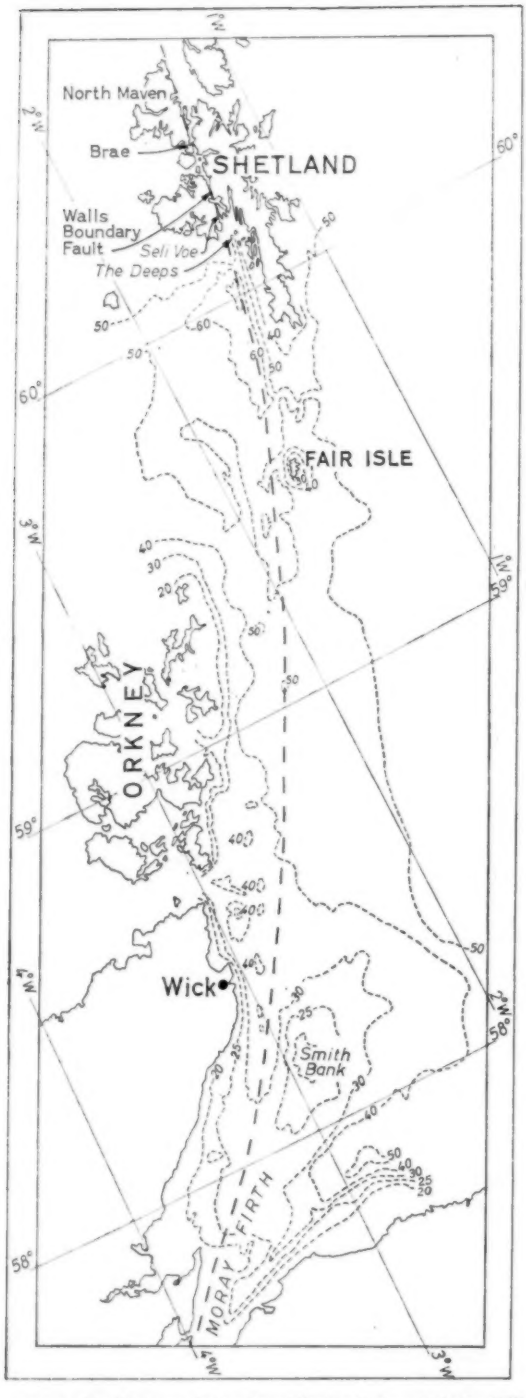

Fig. 1. Possible course for the Great Glen Fault through Shetland to the edge of the continental shelf north of Shetland

farther north it crosses the isthmus at Brae and can then be traced intermittently up the east coast of North Maven.

In the region between Seli Voe and Aith Voe, Old Red Sandstone intruded by granite lies to the west of the fault and gneisses and granite to the east. Finlay[3], and the Survey[4] report shattering, shearing and mylonitization on an extreme scale in the Old Red Sandstone and the granite, up to distances of more than a mile to the west of the fault. There is much shearing of the metamorphic rocks up to a mile to the east of the fault. At Brae, where the fault crosses the isthmus, it lies in a half-mile wide belt of intense shearing and shattering. On the east coast of North Maven, typical pegmatitic granite of the Graven Intrusive Complex of Delting[5] which is on the east side of the fault is in contact with low-grade schists and phyllites on the west side of the fault. About a mile farther west these metamorphic rocks are separated from a granite by a parallel fault. Intense shattering and shearing are associated with these faults.

The Walls Boundary Fault seems to be one of the most powerful dislocations in Shetland. No correlation across it seems possible; there is little similarity between either the metamorphic or the Old Red Sandstone rocks on either side of it. To the east, the Old Red Sandstone contains no contemporaneous or intrusive igneous material, while to the west it contains great thicknesses of volcanic ashes and lavas and is cut by a series of late granites. The Old Red Sandstones cut by the Walls Boundary Fault are considered by Finlay[3] to be of Lower Old Red Sandstone age. This indicates an age later than Lower Old Red Sandstone for a displacement of major proportions on the fault, and agrees with the findings of Kennedy for the Great Glen Fault.

North of Shetland the sea-floor is rather flat and featureless for some distance. However, the 100-fathom contour shows a small southward invagination on the strike of the projected fault if it continues its anticlockwise swing in strike (Fig. 2). To the north of the 100-fathom contour on the continental slope is a very large-scale feature of a type which would be expected if the continental slope itself had been intersected and offset by a sinistral tear fault with a displacement of about 50 miles.

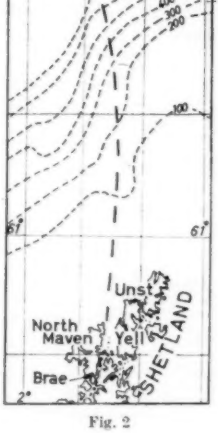

Fig. 2

The soundings on which Figs. 1 and 2 are based and several of the contours have been reproduced from British Admiralty Charts Nos. 115, 2182*c*, 2339, 3057, 1119 and 1118*b* with the permission of the Controller of H.M. Stationery Office and of the Hydrographer of the Navy, for the usual fee.

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[1] Kennedy, W. Q., *Quart. J. Geol. Soc. Lond.*, **102**, 41 (1946).
[2] *British Admiralty Charts*, 1118*b*, 1119, 2182*c*, 2339, 3057, 115.
[3] Finlay, T. M., *Trans. Roy. Soc. Edin.*, **56**, 671 (1930).
[4] *Summaries Prog. Geol. Surv. Great Britain for 1932*, Pt. 1, 77 (1933).
[5] Flinn, D., *Quart. J. Geol. Soc. Lond.*, **110**, 177 (1954).

CHEMISTRY

Bond-Lengths in Solid Helium

RECENTLY, Vignos and Fairbank[1] reported the existence of a new solid phase of helium-4 which exists in a narrow range of pressure between 1·45° K. and 1·78° K. They have designated the new solid as the γ-phase. Its density is less than that of the α-phase (hexagonal closest packing) of helium with which it is contiguous. It is known[2,3] that in helium-3 at a temperature almost as low, there is a phase transition in the solid from hexagonal closest packing to a less-dense phase which is body-centred cubic. Reasoning by analogy, the authors believe the new phase will be found to have the body-centred cubic, *A*2, structure.

In 1947 Pauling[4] developed an equation which has been successful for treating covalent and metallic bonds and for estimating their change in length on a change in co-ordinating number. Thus, for a proposed change in structure, one could in many cases compute the molar volume of the new structure from the cell constants of the old one. The equation is given in the form :

$$R(1) - R_n = 0{\cdot}300 \log n$$

Here $R(1)$ is the single bond-radius of the element, R_n the radius along a bond direction in a structure, and n is the bond number. Furthermore, n equals v/N, where v is the valence and N is the number of bonds.

In the hexagonal closest-packed structure, each atom has twelve nearest neighbours and in the *A*2 structure eight. Because of the fewer neighbours in the cubic structure, the bond-radii will be shorter even though the molar volume may be greater. If bonds were formed with only the nearest neighbours, the decrease in bond-radius would be:

$$\Delta R = R_n(12) - R_n(8) = 0{\cdot}300 \log 12/8 = 0{\cdot}053 \text{ Å}.$$

However, in the *A*2 structure, there are six more neighbours at a distance only slightly longer than that of the nearest eight, and therefore some of the bond-forming power of the atom is used to make bonds with them. This results in the bond-number of the shorter bonds being less than $v/8$ and the correction in bond radius becoming less than 0·053 Å. Thus in the case cited by Pauling[4], of zirconium, which undergoes a phase transition of the kind discussed here, the bond-number of the stronger bonds is near $v/9{\cdot}24$ and ΔR is observed to be 0·037 Å.

Although the bonds in solid helium are mainly van der Waals, it is nevertheless instructive to apply Pauling's equation to the known phase change in helium-3. The molar volumes of helium-3 at 2·805° K. at the hexagonal-cubic transition line as deduced from the results of Grilly and Mills[2] are 19·088 c.c. and 19·206 c.c. respectively. From this one obtains $R_n(12) = 1{\cdot}776$ Å., $R_n(8) = 1{\cdot}730$ Å., and $\Delta R = 0{\cdot}046$ Å. The observed ΔR of 0·046 Å. corresponds to a bond-number of $v/8{\cdot}43$. This value of the bond-number is larger than that observed for zirconium partly because the unit cell of helium is larger and partly because the van der Waals forces have a short range. One would not expect these forces to form proportionally as strong a bond with the six farther neighbours as would be the case with structures having covalent or metallic bonding.

Returning to helium-4, if the new phase has the cubic body-centred structure as is supposed, one can compute the change in molar volume during the solid–solid transition. Referring again to the results of Grilly and Mills[2], the molar volume of solid helium-4 under a pressure of 29·88 kgm./cm.² (near 1·723° K.) is 20·742 c.c. The method used in the experimental determination indicates that this is probably the volume of the hexagonal phase (Mills, R. L., and Grilly, E. R., personal communication). In accordance with this, the bond-radius, $R_n(12)$, of 1·826 Å. is obtained. If one uses as a first approximation the correction in bond-radius of 0·046 Å. that occurs in helium-3, then $R_n(8)$ will be 1·780 Å. The latter distance gives a molar volume for the cubic phase of 20·929 c.c. and a ΔV of transition to this form of 0·187 c.c./mol.

Swenson[5] reports the molar volume of solid helium-4 at 28·06 kgm./cm.² pressure (1·6° K.) to be 20·95 c.c. If this solid is also in the hexagonal form, then one obtains 0·189 c.c./mole as the ΔV of transition. If, however, the solid has the *A*2 structure, then the ΔV of transition would be higher by only 0·014 c.c./mole.

Preparations are now under way at this Laboratory to determine the crystal structure of the new solid form.

This work was carried out under the auspices of the U.S. Atomic Energy Commission.

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[1] Vignos, J. H., and Fairbank, H. A., *Phys. Rev. Letters*, **6**, 265 (1961).
[2] Grilly, E. R., and Mills, R. L., *Ann. Phys.*, **8**, 1 (1959).
[3] Schuch, A. F., Grilly, E. R., and Mills, R. L., *Phys. Rev.*, **110**, 775 (1958).
[4] Pauling, L., *J. Amer. Chem. Soc.*, **69**, 542 (1947).
[5] Swenson, C. A., *Phys. Rev.*, **79**, 626 (1950).

Thermo-electric Phenomena in Electrodialysis Systems

ALTHOUGH several authors, notably Staverman[1] and Spiegler[2], have applied the methods of the thermodynamics of the steady-state to the treatment of electrodialysis processes, little or no attention has been given to the possible existence of heat fluxes in electrodialysis experiments. Of course, very useful relationships can be obtained between the flow of charge and the flow of matter through an appropriate treatment of an isothermal experiment, but we cannot say that we have described the system unless we consider the possibility of heat flows induced by these transport processes.

The argument in favour of this point of view may be roughly summarized as follows. We should like to find an expression for the rate of entropy genera-

tion, and if $\bar{S}$ is the entropy current in the steady system then:

$$\dot{S} = \nabla.\bar{S} = \frac{1}{T}\nabla.\bar{Q} + \bar{Q}.\nabla\left(\frac{1}{T}\right)$$

and by an argument based on the existence of local equilibrium within infinitesimal elements of the system:

$$\nabla.\bar{Q} = -\Sigma_i \nabla\mu_j.\bar{J}_j$$

where μ's are the total potentials (chemical, electrical, etc.) and the $\bar{J}$'s are currents of matter or electricity. A more complete discussion of this line of reasoning has been given by Callen[3]. In writing out a complete expression for the various currents that may exist in an electrodialysis system we must deal in the simplest case with at least four items: salt flow, solvent flow, electric current and a heat current. In an electrochemical system, it is convenient to formulate these flows in terms of the ions, the solvent and the heat. The electric current is, of course, the sum of individual ionic flows multiplied by the appropriate ionic charges.

Flow of positive ions $\Phi_1 = -L_{11}\frac{1}{T}\mathcal{F}\mu_1 - L_{12}\frac{1}{T}\mathcal{F}\mu_2 - L_{13}\frac{1}{T}\mathcal{F}\mu_3 + L_{14}\mathcal{F}\left(\frac{1}{T}\right)$

Flow of negative ions $\Phi_2 = -L_{21}\frac{1}{T}\mathcal{F}\mu_1 - \ldots\ldots\ldots + L_{24}\mathcal{F}\left(\frac{1}{T}\right)$

Flow of solvent $\Phi_3 = -L_{31}\frac{1}{T}\mathcal{F}\mu_1 - \ldots\ldots\ldots + L_{34}\mathcal{F}\left(\frac{1}{T}\right)$

Flow of heat $\Phi_4 = -L_{41}\frac{1}{T}\mathcal{F}\mu_1 - L_{42}\frac{1}{T}\mathcal{F}\mu_2 - L_{43}\frac{1}{T}\mathcal{F}\mu_3 + L_{44}\mathcal{F}\left(\frac{1}{T}\right)$

Elimination of all terms in ∇T simplifies the situation but clearly does not guarantee that $\Phi_4 = 0$.

I have begun a study of the coupling between the electrical currents and the possible heat flows in electrodialysis systems using the apparatus indicated schematically in Figs. 1 and 2. In the first experiments, made with the apparatus shown in Fig. 1, measurement of the voltage developed between the isothermal probes showed that an electrical current did indeed flow under the influence of a temperature gradient, and the sign of the potential indicated that, contrary to my initial expectation, the salt was becoming concentrated in the warmer part of the loop. Unfortunately, this particular apparatus is not suitable for obtaining quantitative relationships with thin-film ion exchange membranes.

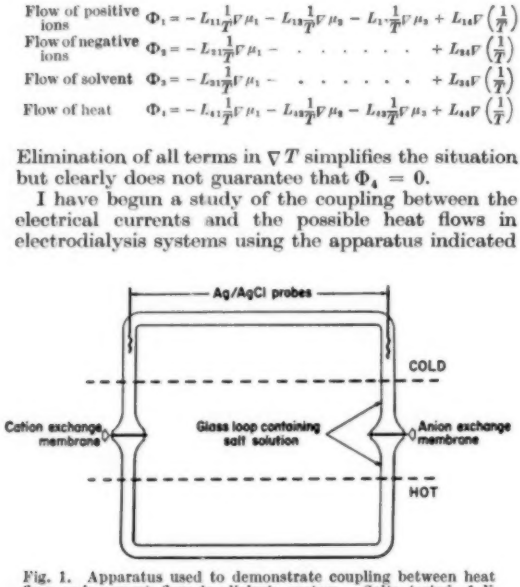

Fig. 1. Apparatus used to demonstrate coupling between heat flow and current flow in dialysis system. Salts tested, 1 *N* sodium chloride and 1 *N* lithium chloride. Left electrode is invariably negative when temperature is controlled as shown

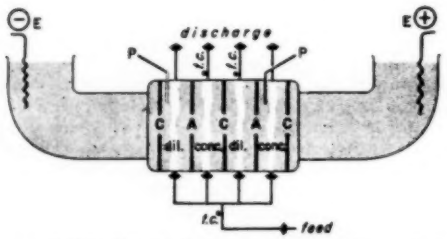

Fig. 2. Apparatus used to measure heat flow between diluting and concentrating compartments. *P*, Probes; *C*, cation exchange membrane; *A*, anion exchange membrane; *E*, power electrode (heavy silver/silver chloride mesh); *t.c.* ●, thermocouple

Some quantitative results have, however, now been obtained with the apparatus indicated schematically in Fig. 2. Electrodialysis experiments have been conducted in this system under adiabatic conditions. The liquid circulation-rates through the various compartments are made as nearly equal as possible; and, with the electric current and the circulation-rate adjusted so that there is little or no change in the concentration of the fluid as it passes through the alternating diluting and concentrating compartments, the changes in temperature in the streams indicated are measured. An estimate of the I^2R heating per cell is obtained from the current passed and the voltage developed at the probes. Under the conditions of these experiments, this loss is equally distributed between the cells; and, from this, the flow-rates, and the observed differences in the rise in temperature, we can calculate the amount of heat transferred into or out of a compartment due to transfer of salt across the membranes. The following preliminary results have been obtained:

Salt	Temperature rise: Concentrated stream	Temperature rise: Diluted stream	Heat transferred per faraday
1 *M* Sodium chloride	0·275° C.	0·65° C.	3,030 cal.
1·27 *M* Lithium bromide	0·30° C.	0·50° C.	2,930 cal.
1·27 *M* Lithium bromide	0·10° C.	0·30° C.	3,240 cal.
8·4 *M* Lithium bromide	1·05° C.	1·70° C.	3,420 cal.
8·4 *M* Lithium bromide	1·38° C.	2·30° C.	3,510 cal.

These results are in rough agreement with those that can be calculated more directly from the fluid circulation-rates and the estimated heat capacities of the solutions used. In every case, flow of heat across the membrane is in a direction opposite to the flow of matter. I do not believe that these very substantial heat quantities are due to the existence of terms such as $-T\Delta S\nabla\left(\frac{1}{T}\right)$ (this term results from $\frac{1}{T}\frac{\partial\mu}{\partial T}\nabla T$ in the full expression for $\nabla\mu$) in the formulation that I have used to describe the currents of matter, electricity and heat, because, as has been shown by deBethune[4], the appropriate ΔS here is only of the order of a few cal./mole and in any event in the experiments I have performed $\nabla\left(\frac{1}{T}\right)$ is a very small quantity. The heat effect appearing here must be regarded as the electrochemical analogue of the Peltier effect.

Further work on a more refined apparatus in which we can further minimize thermal gradients is in progress, and a more complete report on the results is planned.

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[1] Staverman, *Trans. Farad. Soc.*, **48**, 176 (1952).
[2] Spiegler, *Trans. Farad. Soc.*, **54**, 1408 (1958).
[3] Callen, *Phys. Rev.*, **73**, 1349 (1948).
[4] deBethune, *J. Electrochem. Soc.*, **107**, 829 (1960).

Crystal and Molecular Structure of Diphenyl

X-RAY diffraction studies of crystalline diphenyl have been described by a number of authors[1–4] but the bond-distances and bond-angles have not been determined as accurately as in other aromatic compounds recently reported[5]. A detailed X-ray investigation of this structure has now been completed, and in view of the relevance of accurate structural information to modern valency theory, some results of this analysis are reported here.

Three-dimensional counter diffractometer data within the range $0 < \frac{\sin\theta}{\lambda} < 0{\cdot}61$ have been refined by the method of least squares. The average estimated standard deviations:

$$\sigma_{xi} = [C_{ii}^{-1}\Sigma\omega\Delta^2/n - k]^{1/2}$$

(where C_{ii}^{-1} is the appropriate diagonal element of the inverse of the 9×9 matrix solved for each atom[6]) are 0·0016 Å. and 0·019 Å. for carbon and hydrogen co-ordinates respectively. The usual residual factor for 540 observed reflexions is 0·038; the space group is shown conclusively to be $P2_1/c$ and the molecule is therefore centrosymmetric and cannot be twisted as it is in the vapour phase[7].

The molecule is not strictly planar; the equation of the least-squares plane through the asymmetric unit[8] is:

$$-0{\cdot}30584\,X + 0{\cdot}55575\,Y + 0{\cdot}77305\,Z' = 0{\cdot}10114$$

while that of the mean molecular plane is:

$$-0{\cdot}30891\,X + 0{\cdot}55579\,Y + 0{\cdot}77810\,Z' = 0$$

(These direction cosines refer to the orthogonal crystal axes a,b,c'.) The root mean square deviations from the ring plane are 0·0035 Å. for carbon atoms (maximum = 0·0049 Å.) and 0·012 Å. for hydrogen atoms (maximum = 0·017 Å.). The observed deviations of the carbons from this plane, while small, are statistically significant ($\chi^2 = 29{\cdot}1$ with 3 degrees of freedom)[9] and are possibly due to intermolecular packing. For the molecular plane, the root mean square deviations are 0·0050 Å. and 0·019 Å. ($\chi^2 = 58{\cdot}3$ with 4 degrees of freedom).

The bond-distances and -angles derived directly from the best least-squares co-ordinates are given with their estimated standard deviations in Fig. 1. These values require correction for the effects of molecular rigid body librations, and analysis[10] of the thermal vibration parameters in terms of such modes leads to the corrected distances, angles and estimated standard deviations given in Fig. 2. The effect of this correction is to increase all the carbon–carbon bond distances and to make all bond-angles more nearly equal to 120°. The deviation of the angles BAF ($\sim 17\sigma$) and ABC ($\sim 10\sigma$) from this value are, however, still highly significant. The difference ($\sim 8\sigma$) between the distances AB and CD is also significant, and the differences AB–BC and BC–CD are just at the significance-level.

Since there are no significant differences between any two chemically identical but crystallographically non-equivalent distances or angles it seems unlikely that the estimated standard deviations are seriously under-estimated. In addition, since the least-squares results indicate no significant displacements of the ortho-hydrogens from a planar trigonal arrangement it appears that hydrogen–hydrogen repulsive interactions, to which the apparent non-planarity of the molecule in the vapour phase[7] has been attributed, cannot seriously affect bond-distances or bond-angles which involve only carbon atoms[14]. The observed deviations of the intra-ring carbon atoms from a regular hexagonal arrangement and the 0·20 Å. perpendicular separation between the planes of the two phenyl rings are probably due to intermolecular crystal forces. The effects of π electron delocalization across the linking bond should be small and, moreover, should increase the intra-ring bond-distances above the benzene value (1·397 Å.) whereas the observed values of CD and BC are shorter than this.

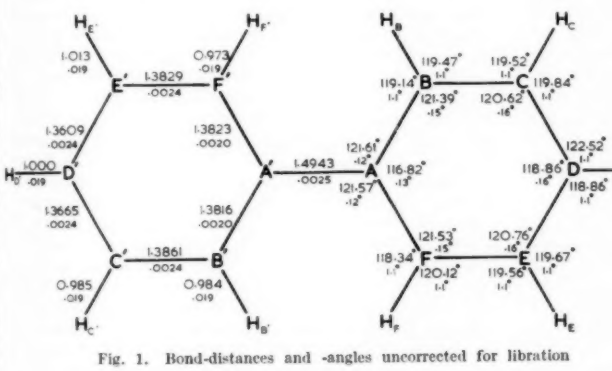

Fig. 1. Bond-distances and -angles uncorrected for libration

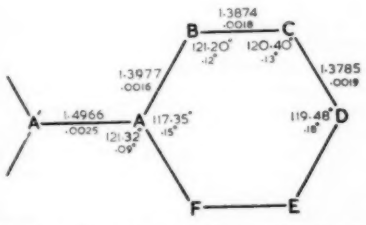

Fig. 2. Bond-distances and -angles corrected for libration

The intra-ring bond-distance is of considerable interest in relation to the recent discussion[11–15] on the effect of hybridization on carbon–carbon distances. The π bond-order derived from simple molecular orbital calculations is 0·37 (ref. 16), and more sophisticated self-consistent field calculations, such as have been reported for butadiene[12], indicate that the correct value is probably considerably less than this. The shortening due to double bonding must therefore be small and the observed distance of 1·497 Å. cannot differ greatly from that of a $C(sp^2) - C(sp^2)$ 'single' bond. This distance is significantly greater than 1·48 Å., which has been suggested[13] for the $C(sp^2) - C(sp^2)$ single bond distance. It is, however, consistent with the proposed distance of 1·51 Å. if, as has been suggested[17], the variation of bond-distance with bond-order is less rapid at low bond-orders than in other regions of the order-distance curve.

The calculations involved in this investigation were carried out on the University of London *Mercury* computer. I am indebted to Mr. O. S. Mills and Drs. J. S. Rollett and R. A. Sparks for making available their programmes for crystallographic calculations.

I am also indebted to Prof. C. J. B. Clews both for suggesting this problem and for his continual encouragement; to Prof. D. P. Craig and Drs. R. Mason and P. Pauling for much helpful discussion; and to the University of Western Australia for providing a research grant.

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1 Dhar, J., *Indian J. Phys.*, **7**, 43 (1932).
2 Dhar, J., *Proc. Nat. Inst. Sci. India*, **15**, 11 (1949).
3 Kitiagorodsky, A. I., *Acta Physiochim. U.R.S.S.*, **21**, 575 (1946).
4 Hargreaves, A., and Hasan Rizvi, S., *Proc. Chem. Soc.*, 122 (1961).
5 For example, Cruickshank, D. W. J., *Acta Cryst.*, **9**, 915 (1956).
6 *International Tables for X-Ray Crystallography*, **2**, 330.
7 Allmeningen, A., and Bastiansen, O., *Det Kgl. Norske Videnskabers Selskabs Skrifter*, No. 4 (1958).
8 Sparks, R. A., Ph.D. thesis, University of California at Los Angeles (1958).
9 Cramer, H., *The Elements of Probability Theory* (John Wiley and Sons, Inc., New York, 1955).
10 Cruickshank, D. W. J., *Acta Cryst.*, **9**, 754, 757 (1956).
11 For example, Goodwin, T. H., *J. Chem. Soc.*, 4851 (1960).
12 Mulliken, R. S., *Tetrahedron*, **6**, 68 (1959).
13 Dewar, M. J. S., and Schmeising, H. N., *Tetrahedron*, **5**, 105 (1959).
14 Cruickshank, D. W. J., and Sparks, R. A., *Proc. Roy. Soc.*, A, **258**, 270 (1960).
15 Brown, M. G., *Trans. Farad. Soc.*, **55**, 694 (1959).
16 Coulson, C. A., and Longuet-Higgins, H. C., *Proc. Roy. Soc.*, A, **195**, 188 (1948).
17 Mason, R. (private communication).

Pyrene Dimers

The striking change in the fluorescence spectrum from pyrene in benzene as a function of the pyrene concentration was studied by Förster and Kasper[1]. They concluded that the violet emission is produced by monomolecular pyrene while the blue emission is from transient dimers. Schmillen[2] has observed similar changes in the fluorescence spectrum from pyrene in solid hydrocarbons and has reported decay times of 8·9–9·9 nsec. for monomolecular pyrene and approximately 89 nsec. for pyrene complexes. The true value of the lifetime of pyrene dimers in liquid solutions has recently been the subject of discussion[3,4]. This communication reports the results of studies of changes in the fluorescence lifetime and in the emission spectrum of pyrene in paraxylene and in cyclohexane for various concentrations of pyrene.

Paraxylene and cyclohexane were purified with two vacuum distillations, but the pyrene (which, like the solvents, was obtained from the Eastman Kodak Co.) was used without further purification. The sample solutions were flushed with nitrogen before each measurement to obviate oxygen quenching and were handled with caution since pyrene is listed[5] as a carcinogen.

Decay times were measured using apparatus[6] which produced a pulsed beam of electrons of 75 keV. energy to excite the fluorescence, and a 1P28 photomultiplier and associated equipment for detecting and graphically recording, the shape of the emitted light pulse. Each component in the fluorescence emission was studied by interposing either a 9863 Corning filter or a Wratten 2 series VI filter to pass the violet and blue components, respectively. This equipment can measure decay times conveniently over a range of 2–200 nsec. Longer components in the decay could be observed but not accurately determined.

Table 1. Measured Decay Times

Solute	Solute concentration (gm./l.)	Filter type	Decay time (nsec.)
—	Pyrene crystals	Wratten 2 (blue)	91 ± 5
Xylene	0·1	Wratten 2 (blue)	91 ± 10
Xylene	0·5	Wratten 2 (blue)	91 ± 10
Cyclohexane	0·5	Wratten 2 (blue)	81 ± 10
Xylene	0·1	9863 (violet)	91 ± 5
Xylene	0·5	9863 (violet)	70 ± 4
Cyclohexane	0·5	9863 (violet)	65 ± 4

The measured decay times for several sample solutions are listed in Table 1. The two arbitrary values of the concentration of pyrene in xylene, 0·1 and 0·5 gm./litre, are chosen because with the former concentration the blue component in the emission spectrum is starting to assume its characteristic shape whereas, in the latter case, the blue component is quite prominent. No decay-time data are reported for a 0·1 gm./litre pyrene–cyclohexane solution as the fluorescence yield is too small to be measured accurately.

It is noted in Table 1 that the value for the violet component is, to a small extent, dependent on the solute concentration. The decrease in the decay time of the violet component with increasing solute concentrations coincides with the decrease in the intensity of the emission yield. For the same concentration (0·5 gm./l.) of pyrene, the value of the decay time and emission intensity of the violet component is less when the solvent is cyclohexane rather than xylene. These observations are consistent with one of two mechanisms which can explain the formation of the anomalous blue component at the expense of the violet component: (1), the existence of transient dimers or (2), energy transfer from monomeric pyrene to permanent aggregations.

Moreover, it was observed that the time taken for the blue component to rise is at least an order of magnitude longer than that of the violet component, and that it becomes shorter with increasing solute concentration. Since a long time in rising is indicative of a delay in the emission process, this observation supplies further evidence for either of the two postulates.

Fluorescence spectral measurements[6] were made to compute the energy-transfer parameter ε in an attempt to ascertain the existence and proliferation of permanent aggregations. A thin (0·05 mm.) sample of solution was excited with 265-mμ radiation from a Bausch and Lomb monochromator and the transmitted emission spectra from both solvent and solute were recorded by a Beckman *DK*-2 spectrophotometer. The exciting light is almost completely absorbed by the solvent. Some of this excitation energy is re-emitted and a portion is transferred to solute molecules. From the change in the intensity of the solvent yield the value of ε could be determined[4] and over the concentration range of 0·05–2 gm./l., ε, as a fraction of unity, could be expressed to a good approximation by the relationship $\varepsilon = 6{\cdot}8\,c/(1 + 6{\cdot}8c)$, where c is the solute concentration in gm./l. If, following Cohen and Weinreb[7], one interprets a linear dependence of c/ε versus c as an indication that only one level in the solvent molecule and only one species of acceptor molecule are active in the energy-transfer process, one must conclude that if there were permanent aggregations of pyrene they do not noticeably contribute to the deactivation of the excited solvent molecules.

Although these results provide supporting evidence for the assumption of transient dimers as postulated

by Förster and Kasper[1], they are not sufficiently conclusive to eliminate other interpretations.

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[1] Förster, Th., and Kasper, K., *Z. Elektrochem.*, **59**, 977 (1955).
[2] Schmillen, A., *Naturforsch.*, **16**, a, 5 (1961).
[3] Stevens, B., and Hutton, E., *Nature*, **186**, 1045 (1960).
[4] Parker, C. A., and Hatchard, C. G., *Nature*, **190**, 165 (1961).
[5] *Dorland's Illustrated Medical Dictionary* (W. B. Saunders Co., 1960).
[6] Berlman, I. B., *J. Chem. Phys.*, **33**, 1124 (1960).
[7] Cohen, S. G., and Weinreb, A., *Proc. Phys. Soc.*, B**69**, 593 (1960).

2,6-Difluoropyridine

As part of a programme involving the investigation of general laboratory methods for the preparation of fluoropyridines[1], we wish to report the synthesis of 2,6-difluoropyridine. The only other recorded example of a pyridine containing more than one nuclear fluorine is pentafluoropyridine[2,3], which was prepared by methods best suited for the synthesis of perfluoro-aromatic compounds.

The present report describes the synthesis of 2,6-difluoropyridine from starting materials readily obtainable by standard laboratory procedures. Conversion of methyl 6-fluoropicolinate[4] (60·0 gm.) to 6-fluoropicolinic hydrazide (51·2 gm.), m.p. 120·5–121° (found: C, 46·7; H, 4·0; $C_6H_6FN_3O$ requires C, 46·5; H, 3·9 per cent), with hydrazine in methanol, followed by a modified Curtius-type degradation, gave 2-amino-6-fluoropyridine, m.p. 58–59° (found: C, 53·6; H, 4·3; N, 24·9; $C_5H_5FN_2$ requires C, 53·6; H, 4·5; N, 25·0). As it is known that fluorine atoms adjacent to aromatic ring nitrogens undergo facile hydrolysis in acid solution[5], it was found advisable to reverse the normal addition procedure of the Curtius degradation. Consequently, addition of *N* hydrochloric acid to a stirred slurry of sodium nitrite dissolved in water, 6-fluoropicolinic hydrazide (20·0 gm.), and ether gave 6-fluoropicolinic azide. This was decomposed in warm carbon tetrachloride to crude 6-fluoropicolinic *iso*cyanate, which was hydrolysed directly to 2-amino-6-fluoropyridine (7·2 gm.) with 50 per cent aqueous acetic acid. By use of Roe's modification of the Schiemann reaction[6], 2-amino-6-fluoropyridine (10·0 gm.) was converted to 2,6-difluoropyridine (2·8 gm.), b.p. 124·5° (743 mm.), n_D^{25} 1·4349 (found: C, 52·2; H, 2·6; N, 12·1; $C_5H_3F_2N$ requires C, 52·2; H, 2·6; N, 12·2).

The infra-red spectrum of 2,6-difluoropyridine in carbon tetrachloride, obtained with a Perkin–Elmer model 21 spectrophometer equipped with sodium chloride optics, showed absorptions at 1,607, 1,592 and 1,493 cm.$^{-1}$, providing evidence for a pyridine nucleus. Additional absorptions at 3,110 and 717 cm.$^{-1}$ contribute support for the proposed structure of this compound.

2,6-Difluoropyridine is exceptionally volatile and shows the same solubility characteristics as 2-fluoropyridine. In contrast to pentafluoropyridine, it does exhibit some basic properties in 50 per cent ethanol when titrated against 0·01 *N* hydrochloric acid. However, no hydrochloride was formed in ethereal hydrogen chloride and no picrate formed in ethanol.

An attempt to prepare 2,3-difluoropyridine from methyl 2-fluoronicotinate (10·0 gm.), b.p. 101° (10 mm.), n_D^{25} 1·4978 (found: C, 54·4; H, 3·9; N, 9·1; $C_7H_6FNO_2$ requires C, 54·2; H, 3·9; N, 9·0) by a similar route failed because hydrazine was found to react preferentially with the fluorine rather than the ester moiety giving methyl 2-hydrazinonicotinate (1·3 gm.), m.p. 106–106·5° (found: C, 50·4; H, 5·4; N, 25·4; $C_7H_9N_3O_2$ requires C, 50·3; H, 5·4; N, 25·1).

A more detailed report of this and related work will be published elsewhere.

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[1] Finger, G. C., and Starr, L. D., *J. Amer. Chem. Soc.*, **81**, 2674 (1959).
[2] Stacey, P. M., and Tatlow, J. C., *Nature*, **186**, 231 (1960).
[3] Haszeldine, R. N., *et al.*, *Proc. Chem. Soc.*, 211 (1960).
[4] Roe, A., Cheek, P. H., and Hawkins, G. F., *J. Amer. Chem. Soc.*, **71**, 4152 (1949).
[5] Miller, W. K., Knight, S. B., and Roe, A., *J. Amer. Chem. Soc.*, **72**, 4765 (1950).
[6] Roe, A., and Hawkins, G. F., *J. Amer. Chem. Soc.*, **69**, 2443 (1947).

RADIATION CHEMISTRY

Polarization induced by Radiation in 'Perspex'

IF, after irradiation of a thick piece of 'Perspex' with a sufficient number of electrons of a few MeV., the specimen is knocked with a sharp tool, a rather intense 'lightning flash' is seen and a tree-like crack pattern occurs. The foot of the stem is situated at the point which was struck. Sometimes the discharge occurs spontaneously towards any disturbance at the surface of the 'Perspex' block.

As the irradiated sample is thicker than the range of the electrons, it is clear that these electrons are stopped in the 'Perspex', mainly at depths corresponding to the more or less straight sloping part of the absorption curve. The 'Perspex' being a good insulator, negative space charge builds up in the sample, and when the dose of radiation is sufficiently high, a discharge can occur.

Gross[1] has already studied this phenomenon in some detail, but some of his statements do not agree with my observations. However, I wish only to report here on another property of the irradiated specimen.

I have bombarded several identical polished 'Perspex' blocks 13 mm. × 38 mm. × 47 mm. with $1·2 \times 10^{14}$ electrons of 2·5 MeV. The current of the linear accelerator was held at 0·1 μamp. The beam entered the blocks in the middle of one of the largest faces. The blocks were examined with a polariscope with convergent light, the specimen being placed so that one of the largest faces was viewed. Before irradiation, no interference pattern was observed. Immediately after irradiation, an interference pattern could be seen that behaved as if the 'Perspex' block was a uniaxial double refractive crystal, the optical axis coinciding with the direction of the beam. One could observe a circular isochromate and the well-known dark cross, each line of which corresponds to the direction of the polarizer and analyser. This picture disappeared very quickly at first, but more slowly later on. After an hour, it was scarcely visible and after two hours it had disappeared completely, although it was still possible to create some cracking by knocking the sample. If the specimen was knocked when the interference pattern was still strong, the pattern disappeared nearly completely. From this we may conclude that in the circumstances considered

the optical properties of the 'Perspex' bombarded with electrons is mainly due to an electrical effect, the remaining part being due probably to mechanical strain caused by the non-homogeneous increase of the temperature. It seems evident that something like the Kerr electro-optic effect is occurring. The charges in the 'Perspex' are more or less situated in planes perpendicular to the original direction of the beam, causing an electric field in that direction and giving the 'Perspex' the property of behaving as a uniaxial crystal with its axis along the electric field.

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[1] Gross, B., *Phys. Rev.*, **107**, 368 (1959); *J. Polymer Sci.*, **27**, 135 (1958).

BIOCHEMISTRY

Hexosamine and Sialic Acid Contents in Cells

Few data are available on the determination of glycoproteins containing sialic acid in tumour tissues, although the presence of an acid mucopolysaccharide in some kinds of tumour tissue has been reported. We determined the hexosamine and sialic acid contents in tumour cells, the HeLa cells and *C3H* ascitic hepatoma cells of different types, comparing them with those of non-malignant cells. The liver cells of *C3H* mice, and *FL* cells obtained by a tissue culture were used as non-malignant cells. The liver cell is generally considered to be a good example of the basic cell type. The types of the *C3H* ascitic hepatoma used were *MH* 134, *MH* 129 *P* and *MH* 129 *F*[1], which were inoculated into (*C3H*/*He*N*dd*) F_1 hybrid mice, subcutaneously or intraperitoneally. The cells from solid tissue were prepared by use of a tissue presser. The cells were dried with acetone after washing with saline solution. The results of the determination are summarized in Table 1.

The sample used for the sialic acid determination was examined, and the absorption spectrum of the coloured solution which was produced by the resorcinol reagent was read; two peaks of absorption, with maxima at 450 and 580 mμ were obtained. This coincides with the results for sialic acid reported by Svennerholm[4]. From these results no significant difference in hexosamine and sialic acid contents was found between tumour cells and non-malignant cells. Prodi[5] reported the hexosamine contents of liver in rat, rabbit and guinea pig presenting values of 436, 408 and 405 γ/100 mgm. of dry weight. These results are only a little higher than the values obtained in *C3H* mouse liver. Hexosamine and sialic acid contents of the intact liver cells of the mice with intraperitoneally inoculated tumours were almost equal to the normal values, although the serum-level of sialic acid in mice with the same tumour was remarkably high[6]. Thus it was found that glycoprotein containing sialic acid is not stored in tumour cells as well as in the liver cells of tumour-bearing animals, even if the liver were a site of synthesis of these glycoproteins[7]. Furthermore, it may be concluded that tissue destruction is not a direct origin of the increase of serum glycoproteins in tumour-bearing subjects. The presence of a certain kind of substance which stimulates the production of sialic acid-containing substances in tumour-bearing animals is assumed. This assumption is partly based on the finding of the excretion of so-called 'κ-mucopolypeptide', which was stated to be a tumour-specific urinary mucopolypeptide[8].

Table 1. Sialic Acid and Hexosamine Contents of Cells

	Nitrogen	Hexosamine		Sialic acid	
	mgm./100 mgm. dry weight	mgm./100 mgm. dry weight	μgm./mgm. nitrogen	mgm./100 mgm. dry weight	μgm./mgm. nitrogen
FL cell	10·8	0·41	38	0·36	33
HeLa cell	12·0	0·37	31	0·46	38
Tumour cell					
MH 134 (i.p.)	12·9	0·48	37	0·26	20
MH 134 (s.c.)	14·4	0·42	29	0·36	25
MH 129*F* (i.p.)	14·1	0·40	28	0·36	26
MH 129*P* (i.p.)	14·2	0·36	25	0·32	23
Liver cell of *C3H* mice					
Normal	11·8	0·32	27	0·31	26
MH 129*F* bearing	12·6	0·33	26	0·33	26
MH 129*P* bearing	13·0	0·39	30	0·33	25

i.p., Intraperitoneally inoculated tumour; s.c., subcutaneously inoculated tumour.
Hexosamine was determined according to Boas (ref. 2), and sialic acid according to Svennerholm (ref. 3) using the orcinol reagent.

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[1] Sato, H., Kawashima, Y., Kanno, K., Munakata, H., and Saito, T., *Fukushima J. Med. Sci.*, **5**, 155 (1958).
[2] Boas, N. F., *J. Biol. Chem.*, **204**, 553 (1953).
[3] Svennerholm, L., *Acta Chem. Scand.*, **12**, 547 (1958).
[4] Svennerholm, L., *Biochim. Biophys. Acta*, **24**, 604 (1957).
[5] Prodi, G., *Proc. Soc. Exp. Biol. Med.*, **88**, 605 (1955).
[6] Turumi, K., Nagai, Y., Kimura, A., Kawashima, Y., and Sato, H., *Gann*, **50** (Supp. 76, 1959).
[7] Werner, I., *Acta Physiol. Scand.*, **19**, 27 (1950).
[8] Masamune, H., Hakomori, S., and Sugo, T., *Tohoku J. Exp. Med.*, **69**, 383 (1959).

Polarographic Examination of Sialic Acids

We have investigated systematically the polarographic wave of neuraminic acid derivatives with a dropping-mercury electrode ($m = 2{\cdot}86$ mgm./sec., $t = 2{\cdot}98$ sec.). The existence of a sialic acid wave was anticipated from the fact that structurally related substances such as pyruvic acid[1,2] and fructose[3,4] give well-defined polarographic waves. Three type of waves could be observed in N-acetylneuraminic acid solution (NANA): (*a*) a hydrogen wave in 0·1 *M* LiCl or in 0·01–0·1 *M* tetramethylammonium bromide (TMABr) under *p*H 7. The apparent half-wave potential is − 1·65 V. (s.c.e.) in 0·1 *M* TMABr and − 1·48 V. in 0·01 *M* TMABr. There is a linear relationship between the height of the wave and the square root of the height of the mercury column up to 45 cm.; and, under defined conditions,

Table 1. CHARACTERISTICS OF THE WAVES OBSERVED IN NANA SOLUTIONS AS COMPARED WITH THE WAVES OF PYRUVIC ACID AND OF FRUCTOSE

Substance	Condition or pre-treatment	30° C. Half-wave potential, V.	30° C. Height μamp./μmole	60° C. Height μamp./μmole	$\frac{h60°}{h30°}$	Per cent/degree*
Pyruvic acid	TMAOH 0·01 *M*	−1·80	77·6	91·4	1·29	0·59
Fructose	LiCl 0·1 *M*	−1·90	21·0	61·5	2·93	6·4
Fructose	TMAOH 0·01 *M*	−1·95	25·0	66·5	2·65	5·5
NANA	TMAOH 0·01	−1·96	18·3	81·8	4·47	11·6
NANA	Heated 10 min. in TMAOH 0·01 *M*	−1·65				
		−1·95	53·8	74·3	1·38	1·27
NANA	Kept frozen freshly thawed	−1·87	33·0			
NANA	Same sample kept 1 hr. at room temp.	−1·82	26·0			
NANA	Same sample kept 2 hr. at room temp.	−1·86	10·0			

* Temperature coefficient of the wave in per cent change per degree, relative to the wave at 30° C.

a linear relationship between NANA concentration and the height of the wave. Under identical conditions, pyruvic acid ($4·05 \times 10^{-4}$ *M* in 0·01 *M* TMABr, under nitrogen) gives a hydrogen wave of the same height (36·7 μamp.) as NANA (39 μamp.). The slope of the straight line in which the height of the wave is plotted as the function of concentration of NANA is 0·3 μamp./μgm./ml. or 92·7 μamp./μmole/ml. (at 30° C. in 0·01 *M* TMABr). There is a marked sensitivity of the wave to ionic strength and also to the presence of oxygen.

(*b*) When a 0·4 μmole/ml. solution of NANA in 10 μmole/ml. of TMABr is neutralized by stepwise addition of 0·01 *M* TMA-phosphate buffer at *p*H 7, the hydrogen wave decreases and another wave appears on top of it and increases as the *p*H gets closer to neutrality. This second wave has an approximate half-wave potential of −1·75 V. This stepwise change of the two waves is shown by Fig. 1 (polarograms 7, 8, 9). This second wave, given apparently by the anion of NANA, was found in 0·01 *M* tetramethylammonium hydroxide (TMAOH), *p*H 10 (Fig. 1, polarogram 1). The apparent half-wave potential of this wave is independent of the mercury pressure and has a high-temperature coefficient (Table 1). It seems to be a wave controlled kinetically.

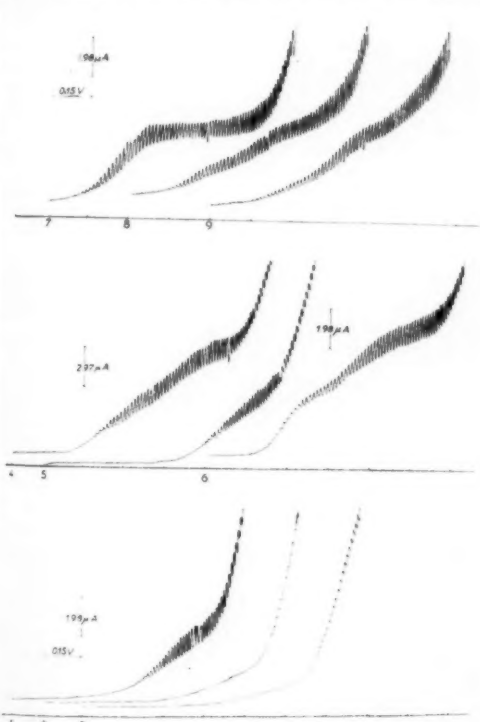

Fig. 1. Polarograms of NANA and related substances. (1) freshly thawed NANA, 1·62 μmole/ml. in TMAOH 0·01 *M*; (2) same as (1) after 2 hr. at room temperature (24° C.); (3) same as (1), after 3·5 hr. at room temperature; (4) pyruvic acid 1·60 μmole/ml. in TMAOH 0·01 *M*; (5) fructose 1·60 μmole/ml. in TMAOH 0·01 *M*; (6) NANA 1·60 μmole/ml. heated 10 min. in TMAOH 0·01 *M* recorded in the same solvent; (7) NANA 0·4 μmole/ml. in TMABr 0·01 *M* (hydrogen wave); (8) Same as (7) after addition of 0·01 *M* $TMAPO_4$, *p*H 7, 0·6 μ*M*; (9) same as (7) with 1·11 μ*M* $TMAPO_4$ buffer, all polarograms recorded at 30° C.

An interesting and as yet unexplained variation of the height of this wave was observed, which depended on the previous treatment of the NANA solution. Solutions adjusted to *p*H 4·5 (*p*H of maximum stability[6]) were kept frozen for several days. Freshly thawed solutions gave a higher wave than solutions kept at 4° C., or freshly thawed solutions allowed to remain for various periods of time at room temperature. The height of the wave decreased with time at room temperature. The phenomenon is apparently reversible within limits, since re-freezing and re-thawing of the material two or three times yielded in each case a wave of the same height as the originally frozen NANA solution. No carboxypyrol-derivatives could be detected by paper chromatography in frozen stored solutions, though small quantities were present in solutions kept at 4° C.

No definite statement can be made as to the nature of the reaction determining the wave-height. A ring-opening, as is the case with glucose or fructose[7], or a dehydration as shown for formaldehyde[8] may be the rate-controlling mechanism for this NANA wave.

(*c*) In heated NANA solutions (10 min. at 100° C. in 0·01 *M* TMAOH solution) a different type of wave was observed, which bears some relationship to the pyruvic acid wave (Fig. 1, polarogram 6). However, no pyruvic acid could be detected on chromatograms and the addition of lactic acid dehydrogenase and reduced diphosphopyridine nucleotide (0·005 *M*) to this solution produced a change in optical density at 340 mμ corresponding to only 10 per cent of the amount of pyruvic acid needed to account for the observed polarographic wave. No 2-carboxypyrol could be detected by paper chromatography in the heated solution. The height of the wave observed in heated NANA solutions is independent of the mercury pressure, but has a low-temperature coefficient (Table 1). Pyruvic acid in 0·01 *M* TMAOH gives a wave which decreases linearly with the square root of the mercury pressure.

More experiments are needed before any analytical application of this polarographic wave can be recommended.

The NANA was prepared from meconium according to the method of Zilliken *et al.*, and kindly provided by Dr. R. J. Winzler. Another sample re-crystallized three times was a gift from Dr. Saul Roseman, University of Michigan.

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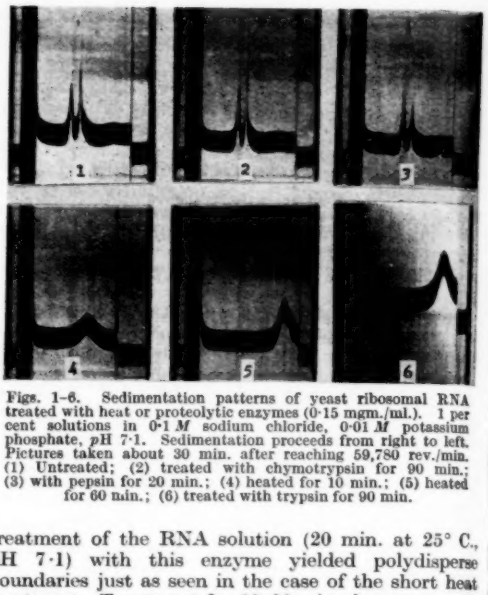
[1] Müller, O. H., and Baumberger, J. P., *Trans. Amer. Electrochem. Soc.*, **71**, 169 (1937).
[2] Brdicka, R., and Wiesner, K., *Coll. Czech. Chem. Comm.*, **12**, 138 (1947).
[3] Heyrovsky, J., and Smoler, I., *Coll. Czech. Chem. Comm.*, **4**, 521 (1932).
[4] Wiesner, K., *Coll. Czech. Chem. Comm.*, **12**, 64 (1947).
[5] Zilliken, F., Braun, G. A., and Gyorgy, P., *Arch. Biochem. Biophys.*, **63**, 394 (1956).
[6] Berggard, I., and Odin, L., *Arkiv för Kemi*, **12**, 581 (1958).
[7] Cantor, S. M., and Peniston, P., *J. Amer. Chem. Soc.*, **62**, 2113 (1940).
[8] Vesely, K., and Brdicka, R., *Coll.*, **12**, 313 (1947).

Sub-unit of Ribosomal Ribonucleic Acid from Yeast

Ribonucleic acid (RNA) isolated by the phenol method from 80*s* ribosomes of yeast was demonstrated to contain two components of 19*s* and 28*s* [1]. In a previous paper[2], it was shown that by heat treatment the high molecular ribosomal RNA could easily be converted to a low molecular RNA with the sedimentation coefficient of about 4*s*. It is also the case for rat liver ribosomal RNA as demonstrated by Takanami[3]. The close agreement of the *s*-value of this heat-treated ribosomal RNA with that of soluble RNA led us to suppose that the 4*s* polynucleotide is the basic unit of all RNA's[2]. In the present work, it has been shown that trypsin, as in the case of heat treatment, can degrade ribosomal RNA into 4*s* sub-units.

Ribosomal RNA used was isolated by the phenol method from *Saccharomyces cerevisiae*, strain Kaneka. In the first series of experiments, the RNA was heated in 0·2 *M* sodium chloride for 10, 20, 40 and 60 min. at 90° C. The heated RNA solutions were observed in a Spinco *E* analytical ultracentrifuge with a schlieren optical technique at once or after dialysis against 0·1 *M* sodium chloride containing 0·01 *M* potassium phosphate buffer at *p*H 7·1. Heating for 10 min. caused a partial degradation of the RNA, giving rather continuously distributed products in the range 4*s*–13*s*, though a component of around 7*s* predominated (Fig. 4). The complete conversion to a 4*s* sub-unit ($S^0_{20} = 4{\cdot}3s$) was achieved by 40 min. heat treatment. Further heating up to 60 min. did not change the sedimentation pattern (Fig. 5).

Next, ribosomal RNA (0·5–1·0 per cent solution in 0·1 *M* sodium chloride–0·01 *M* potassium phosphate) was treated with three kinds of proteolytic enzymes respectively (all 0·15 mgm./ml.), that is, trypsin and chymotrypsin (Worthington Biochemical, United States, 2 × cryst.), chymotrypsin and pepsin (Nutritional Biochemical, United States, 2 × cryst.). The sedimentation analyses were directly made for the digest inclusive of added enzyme. The only effective enzyme in degrading the RNA was trypsin. Brief



Figs. 1–6. Sedimentation patterns of yeast ribosomal RNA treated with heat or proteolytic enzymes (0·15 mgm./ml.). 1 per cent solutions in 0·1 *M* sodium chloride, 0·01 *M* potassium phosphate, *p*H 7·1. Sedimentation proceeds from right to left. Pictures taken about 30 min. after reaching 59,780 rev./min. (1) Untreated; (2) treated with chymotrypsin for 90 min.; (3) with pepsin for 20 min.; (4) heated for 10 min.; (5) heated for 60 min.; (6) treated with trypsin for 90 min.

treatment of the RNA solution (20 min. at 25° C., *p*H 7·1) with this enzyme yielded polydisperse boundaries just as seen in the case of the short heat treatment. Treatment for 60–90 min., however, gave a single boundary of about 4*s* ($S^0_{20} = 4{\cdot}0s$) (Fig. 6), the sedimentation pattern being comparable with that of the 'stable sub-unit RNA' obtained by 60-min. heat treatment. Incubation for up to 90 min. at 25° C. at *p*H 7·1 without added enzyme (Fig. 1), or with chymotrypsin (Fig. 2), or incubation for 20 min. at 25° C. at *p*H 3·0 with pepsin (Fig. 3) did not affect the initial sedimentation pattern very much.

A possibility that a trace of ribonuclease present in the trypsin preparation might be responsible for the observed effect of trypsin is probably excluded by the following tests using pancreatic ribonuclease (the action of ribonuclease having specificity different from pancreatic ribonuclease is still open to question, however). The RNA was incubated at 25° C. for 90 min. with a series of concentration of pancreatic ribonuclease (Worthington Biochemical, United States, cryst.) (0·001–1·0 μgm./ml.) and of trypsin (0·02–0·5 mgm./ml.), and the *s*-value of the main peak was recorded in relation to the concentration of the enzyme used (Table 1). In the case of ribonuclease, the higher the concentration of the added enzyme, the greater was the extent of the RNA degradation, no ultracentrifugally detectable 'end point' of

Table 1. Effects of Pancreatic Ribonuclease or Trypsin on Yeast Ribosomal Ribonucleic Acid

Ribonuclease		Trypsin	
Concentration of enzyme (μgm./ml.)	S_{20}*	Concentration of enzyme (mgm./ml.)	S_{20}*
0·000	19, 28†	0·000	19, 28†
0·001	10·5	0·02	9·50
0·005	7·65	0·10	5·33
0·010	5·11	0·15	3·99
0·025	4·69	0·20	3·93
0·100	4·19	0·40	3·75
0·150	3·10	0·50	3·86
1·000	2·00		

* S_{20} of the main peak, uncorrected.
† S_{20} at zero concentration.
Ribosomal RNA (1 per cent solution in 0·1 *M* sodium chloride, 0·01 *M* potassium phosphate, *p*H 7·1) was incubated with the enzymes for 90 min. at 25° C.

degradation being attained. On the other hand, degradation by trypsin clearly revealed a limitation; 0·15 mgm./ml. of trypsin was sufficient to degrade RNA into 4*s*, and no further change in *s*-value was observed with increasing concentration of trypsin.

An attractive hypothesis from the above results might be that the high molecular ribosomal RNA is composed of relatively stable 4*s* sub-units which are linked together through some peptide-like substance. Acid hydrolysates of the RNA preparation (6 *N* hydrochloric acid, 120° C., 8–24 hr.) were found to give ten amino-acids after two-dimensional paper chromatography or starch column chromatography which have been identified as glycine, glutamic acid, serine, alanine, aspartic acid, amino-butyric acid, lysine, threonine, leucine and valine (roughly decreasing in amount as found). Although the fact that the RNA contains a basic amino-acid, lysine, and no aromatic amino-acid is apparently compatible with the findings that the RNA is susceptible to trypsin, but neither to chymotrypsin nor to pepsin, the amount of lysine detected was too small to account for the number of lysine residue required for the sub-unit linkage. The effect of trypsin on the ribosomal RNA presented in this communication might be related to the degradative action of some non-enzymic proteins on the high molecular RNA from Ehrlich ascites tumour cells recently reported by Brown *et al.*[4].

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[1] Maeda, A., *J. Biochem.* (Tokyo), **48**, 363 (1960).
[2] Osawa, S., *Biochim. Biophys. Acta*, **43**, 111 (1960).
[3] Takanami, M., *Biochim. Biophys. Acta*, **39**, 152 (1960).
[4] Brown, R. A., Ellem, K. A. O., and Colter, J. S., *Nature*, **187**, 509 (1960).

Quantitative Determination of Metadrenaline and Normetadrenaline in Normal Human Urine

Several authors have performed investigations on metadrenaline and normetadrenaline, metabolites of catecholamines, since their discovery by Axelrod[1]. Previous work, however, dealt mostly with the urine of rats[2], in which relatively large amounts of the metabolites are excreted, or the isotope tracer of authentic methoxycatecholamines[3] which can be detected easily by their radioactivity. Metadrenaline and normetadrenaline in normal human urine have never been determined quantitatively, because of their very low concentrations. This communication concerns the method of determination of methoxycatecholamines in urine of normal human beings.

An aliquot (5–10 ml.) of urine is adjusted to *p*H 1·0 with hydrochloric acid, and hydrolysed in a boiling water bath for 1 hr. After cooling in tap water, the urine is adjusted to *p*H 10·0 with sodium hydroxide, saturated with sodium chloride, and extracted with five volumes of ethyl acetate under vigorous shaking for 5 min. After centrifugation, the organic phase is separated by pipetting. The extraction is repeated three times. The extracts are combined, and evaporated under reduced pressure to dryness at a temperature below 60° C. The residue is dissolved in 2 ml. of methanol, and transferred to a small glass tube. This is repeated three times. The methanol extract is evaporated to dryness in the same way. The residue is dissolved in a small amount of methanol.

The methanol extract is placed on Toyo Roshi filter paper No. 51 (5 × 60 cm.), which has been pretreated with pyridine–acetic acid–water (1 : 10 : 89 v/v) just before use, at a distance of 8·5 cm. from the anode end. The paper is subjected to high-voltage paper electrophoresis for 2 hr., using *n*-hexane as refrigerating insulator. Operating voltage is 2,500–3,000 V., current 8–12 m.amp. Metadrenaline migrates about 40 cm. toward the cathode, and normetadrenaline about 42 cm., so that they are separated completely from other substances as well as from each other.

After the paper is dried at 60° C. for 20 min., diazotized *p*-nitroaniline is sprayed on both sides of the paper. Both metabolites react to give a violet colour. The paper is dried again, the bands of methoxycatecholamines are cut into pieces, and eluted with 4 ml. of alkaline methanol (methanol–2 per cent sodium carbonate, 2 : 1 v/v) for more than 1 hr. These eluates are assayed in a Beckman spectrophotometer model *DU* at the wave-length of 520 mμ. The uncoloured part of the paper is treated similarly and serves as a blank.

Table 1. Amounts of Metadrenaline and Normetadrenaline in 24-hr. Urine of Normal Subjects and Various Patients

Subject	Sex	Disease	Metadrenaline (μgm./24 hr.)	Normetadrenaline (μgm./24 hr.)
K. Y.	♀	Healthy	246	239
Y. H.	♂	,,	256	245
A. G.	♂	,,	254	186
K. Y.	♂	,,	172	135
T. K.	♀	,,	257	97
T. C.	♀	,,	125	147
H. S.	♂	,,	225	230
K. M.	♂	,,	104	127
K. O.	♂	Essential hypertension	153	156
J. K.	♂	,, ,,	211	143
S. O.	♂	,, ,,	255	143
S. S.	♂	,, ,,	199	96
S. S.	♀	,, ,,	120	119
M. T.	♂	,, ,,	105	160
S. S.	♂	Graves's disease	143	230
M. H.	♂	,, ,,	138	201
M. S.	♀	,, ,,	182	127
T. S.	♂	Parkinsonism	192	209
G. W.	♂	Primary aldosteronism	231	132
S. I.	♂	Hysteria	416	449
A. I.	♂	Phæochromocytoma	31,400	5,570
J. N.	♀	,,	7,700	8,270

Extraction (using *n*-butanol, *iso*butanol *iso*amyl-alcohol were examined) of the urine was found inadequate, for inorganic salts were extracted together, which interfered seriously with further procedures. The metabolites were not extracted into ethyl ether or ethylene dichloride as efficiently as into ethyl acetate. Purification of *O*-methylated catecholamines with ion-exchange resin was also unsuccessful.

High-voltage paper electrophoresis was found most suitable for the separation of the amines in the extract. Paper chromatography using several solvent systems did not result in good separation of them, as compared with the electrophoretic method.

When the urine was not hydrolysed, the estimated values were much lower than those after hydrolysis. Hydrolysis at approximately *p*H 0 (checked by test paper) resulted in lower yields as compared with that at *p*H 1·0. In accordance with Goodall *et al.*[4],

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incubation of urine with bacterial β-glucuronidase gave rise to no increase in metadrenaline and normetadrenaline values.

The minimal quantity detectable by the method was 0·2–0·3 μgm. for both amines. It was found after repeated estimations that errors of about ± 7 per cent are inevitable in the determination. The overall recovery of the amines added to urine was 80–90 per cent.

Twenty-two urine specimens from normal subjects and various patients were assayed (Table 1). Normal human beings excrete 100–300 μgm. of metadrenaline and almost the same amount of normetadrenaline daily in the urine. Patients with essential hypertension or hyperthyroidism excrete approximately the same quantities of metabolites as normal subjects. Two patients with phæochromocytoma excreted abnormally large quantities of metadrenaline and normetadrenaline.

Our method is relatively simple, reproducible and sensitive enough to estimate such low concentrations of metadrenaline and normetadrenaline as are excreted in the urine of normal human beings.

We are indebted to Dr. Julius Axelrod for his gift of metadrenaline and normetadrenaline.

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[1] Goodall, M., *Pharmacol. Rev.*, **11** (Part 2), 416 (1959).
[2] Bernheimer, H., Ehringer, H., Heistracher, P., and Kraupp, O., *Biochem. Z.*, **332**, 416 (1960).
[3] Axelrod, J., *Science*, **126**, 400 (1957).
[4] Goodall, M., Kirshner, N., and Rosen, L., *J. Clin. Invest.*, **38**, 707 (1959).

Effect of Pituitary Growth Hormone on Cholesterogenesis

It has been reported earlier that injections of anterior pituitary growth hormone into adult female rats cause a decrease in the rate of synthesis of fatty acid and an increase in the rate of cholesterogenesis in the liver[1]. Inverse relationships of this type have also been described as occurring in diabetic rats[2] and in normal rats injected with thyroxine[3]. These results were obtained using labelled acetate as substrate. The availability of labelled mevalonic acid now permits some dissection of the pathway involved.

The rats used were adult females injected with 1 mgm. pituitary growth hormone 6 hr. before killing. After killing, the livers were rapidly removed and slices prepared with the aid of a Stadie–Riggs[4] microtome. 400 mgm. of slices were suspended in 5 ml. Krebs–Ringer bicarbonate buffer containing either 0·6 μc. sodium acetate-1-^{14}C or *dl*-sodium mevalonate-2-^{14}C with no carrier added. The flasks were gassed with an oxygen/carbon dioxide mixture (95 : 5) and were incubated for 3 hr. After this period the slices were collected by centrifugation and then suspended and homogenized in 4 ml. of 5 per cent trichloracetic acid. The precipitate obtained from this was washed with 5 per cent trichloracetic acid and then water, the supernatant being discarded in each case. The precipitate was then extracted with alcohol/ether (3 : 1) and, finally, ether. The combined organic solvent layers were evaporated and the residue re-extracted with petroleum ether (40°–60°). After separation of the phospholipids as the magnesium complexes, the supernatant was saponified and the free cholesterol extracted and precipitated as the digitonide. Fatty acids were then separated after acidifying the supernatant fraction. Samples from each of the separated fractions were counted as infinitely thin layers in a 'Tracerlab' *SC*–16 windowless counter.

The results showing the incorporation of the two radioactive substrates into fatty acids, phospholipids and cholesterol are given in Table 1.

It will be seen that the incorporation of both mevalonate and acetate carbon into phospholipids is reduced in animals treated with growth hormone although, as might be expected, the reduction is much greater in the case of acetate. The incorporation of acetate-carbon into the fatty acid fraction (derived mainly from glyceride and cholesterol esters with some free fatty acid) is also much reduced in the rats treated with growth hormone and to about the same degree as the reduction found in the fatty acids of the phospholipid fraction. However, the incorporation of carbons from mevalonic acid into the ester fatty acids appears to be slightly accelerated in the animals treated with hormone. This somewhat surprising result may mean that the fatty acids formed from mevalonate carbons differ from those derived from acetate.

Finally, it can be seen that while the incorporation of acetate carbon into cholesterol is speeded up by a factor of 4–6 times in rats treated with growth hormone, the rate of incorporation of mevalonate remains unchanged. Thus, the stimulating effect of the hormone on the rate of synthesis of cholesterol

Table 1. Effect of Pituitary Growth Hormone on the Incorporation of Mevalonate-2-^{14}C and Acetate-1-^{14}C into Cholesterol, Phospholipids and Fatty Acids of Liver Slices

Precursor	Condition of animal	Phospholipids		Fatty acids		Cholesterol	
		c./min./flask	Treated/control	c./min./flask	Treated/control	c./min./flask	Treated/control
Mevalonic	Control	215	0·68	1,411	1·27	26,700	0·9
	Hormone-treated	147		1,719		24,418	
Acetate	Control	8,356	0·21	16,619	0·24	1,860	6·1
	Hormone-treated	1,780		3,988		11,311	
Mevalonic	Control	221	0·42	855	1·26	21,120	1·0
	Hormone-treated	92		1,077		21,168	
Acetate	Control	6,782	0·20	14,827	0·20	2,117	4·1
	Hormone-treated	1,356		2,965		8,782	

appears to be located in that part of the pathway between acetyl coenzyme A and mevalonic acid. The earlier part of this pathway, leading to the formation of β-hydroxy-β-methyl-glutaryl co-enzyme A, is common to both cholesterol synthesis and acetoacetate formation. Since it has previously been shown that in rats treated 6 hr. previously with growth hormone the rate of hepatic acetoacetate output is much reduced[5], it would appear that the increased rate of formation of cholesterol is due to a diversion of the β-hydroxy-β-methyl-glutaryl co-enzyme A from acetoacetate formation, and an increased rate of its reduction to mevalonic acid by its reductase. The hormone appears to have no effect on the subsequent condensation of the mevalonate molecules to cholesterol. This finding is in keeping with the conclusion of Bucher, Overath and Lynen[6], who have suggested that the decreased rate of formation of cholesterol in starving animals is due to a reduction in the activity of the β-hydroxy-β-methyl-glutaryl co-enzyme A reductase.

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[1] Greenbaum, A. L., and Glascock, R. F., *Biochem. J.*, **67**, 360 (1957).
[2] Hotta, S., and Chaikoff, I. L., *J. Biol. Chem.*, **198**, 895 (1952).
[3] Fletcher, K., and Myant, N. B., *J. Physiol.*, **144**, 361 (1958).
[4] Stadie, W. C., and Riggs, B. C., *J. Biol. Chem.*, **154**, 687 (1944).
[5] Greenbaum, A. L., and McLean, P., *Biochem. J.*, **54**, 413 (1953).
[6] Bucher, N. L. R., Overath, P., and Lynen, F., *Biochim. Biophys. Acta*, **40**, 491 (1960).

Blockage of Transformation of Tryptophan to Nicotinic Acid by Experimental Hepatitis in Mice

The existence of biochemical lesions in the complicated path from amino-acid to vitamin in man, and in experimental animals suffering from various diseases, has been identified by a number of authors analysing urinary metabolites of tryptophan by means of paper and column chromatography[1–6]. These analyses have shown an accumulation in the urine of metabolites proximal to the lesion.

In the present work we have studied the chromatographic picture of urinary metabolites of the tryptophan–nicotinic acid cycle both in normal mice and in mice with experimental hepatitis induced by mouse hepatitis virus strain 3 (*MHV*-3) at different stages of multiplication, in an attempt to identify the level of a possible biochemical lesion. Since the spontaneous elimination of urinary metabolites of tryptophan is small, we have preferred to follow them after a dose of L-tryptophan.

Mice of Swiss stock, weighing about 15 gm. and fed on Voght and Moller's diet were used. The programme of one of our experiments was as follows: 100 mice were divided into 5 (*A*, *B*, *C*, *D*, *E*) groups of twenty animals. Ten mgm. of L-tryptophan were injected subcutaneously into each animal in order to maintain the normal excretory pattern. After five days, mice from groups *A*, *B*, *C* and *D* were inoculated by the intraperitoneal route with 100 LD_{50} virus *MHV*-3.

To study the excretory pattern of metabolites of the biological degradation of tryptophan in different phases of the multiplication of the virus, a second dose of tryptophan was given to groups *A*, *B*, *C* and *D* at 12, 24, 48 and 72 hr. respectively. The mice in group *E*, not inoculated with the virus, were also given a second dose of tryptophan simultaneously with group *A*.

In the 24 hr. following the injection of tryptophan, the urine from each group of mice was collected in opaque glass vessels containing 2 ml. of 50 per cent glacial acetic acid (1: 1), and was diluted with water to a volume of 5 ml. The urine was then centrifuged. Dalgliesh's[7] method of chromatographic analysis for urinary metabolites in the tryptophan–nicotinic acid cycle was used, but omitting the absorption on carbon and the subsequent treatment with phenol.

The analysis of the urine of the control mice (*E*) injected with L-tryptophan showed the appearance of conjugates of the kynurenines (R_F 0·1–0·15) of kynurenic and xanthurenic acids, of acetylkynurenine and sometimes of free anthranilic acid.

In contrast, the urine of the mice inoculated with the virus showed the presence of all these metabolites (except anthranilic acid) in greater and more constant quantities, and in addition, the presence of kynurenine and of 3-hydroxykynurenine in notable quantities. The chromatographic picture also showed the progressive growth of the metabolic lesion paralleling the aggravation of the viral hepatic lesion.

Other metabolites, such as 3-hydroxyanthranilic acid, have never been observed on normal mice or in mice inoculated with the virus.

From the results obtained, we conclude that in mice with experimental hepatitis caused by the *MHV*-3 virus a biochemical lesion occurs at the level of enzymic transformation of 3-hydroxykynurenine to 3-hydroxyanthranilic acid.

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[1] Altman, K. I., and Miller, G., *Nature*, **172**, 868 (1953).
[2] Boyland, E., and Watson, G., *Nature*, **177**, 837 (1956).
[3] Musajo, L., Benassi, C. A., and Parpaiola, A., *Clin. Chim. Acta*, **1**, 229 (1956).
[4] Quagliariello, E., Auricchio, S., Casale, M., and Tancredi, F., *Boll. Soc. Ital. Biol. Sper.*, **34**, 1918 (1958).
[5] Auricchio, S., Quagliariello, E., Rubino, A., and Vecchione, L., *Ann. Paediat.*, **194**, 129 (1960).
[6] Quagliariello, E., Auricchio, S., Saccone, C., and Sganga, W., *Boll. Soc. Ital. Biol. Sper.*, **35**, 343 (1960).
[7] Dalgliesh, C. E., *J. Clin. Path.*, **8**, 73 (1955).

Sucrases in *Phaseolus vulgaris*

During work on the oligosaccharides of *Phaseolus vulgaris*, L. (namely, verbascose, stachyose, raffinose and sucrose[1–3]—the raffinose family of oligosaccharides[1]), an examination was made of the enzymes catalysing the hydrolysis of these saccharides. α-Galactosidase and sucrase activities were found in the ungerminated and germinated seeds.

The α-galactosidase activity proved to be typical of that described by Courtois *et al.*[4–7] in extracts of coffee and haricot beans and by Pridham[8] in extracts of *Vicia faba*, since it removed galactose from verbascose, stachyose and raffinose *in vitro* to give sucrose. From the higher homologues, verbascose and stachyose, the galactose was removed stepwise

(cf. Courtois *et al.*[4]), producing stachyose and raffinose respectively as intermediates. The α-galactosidase activity was found in extracts of both the embryos and cotyledons in the ungerminated and germinated seeds.

On the other hand, two types of sucrase activity were found. These differed in both properties and occurrence; one activity appeared to resemble the invertases previously demonstrated in extracts of other seeds[9–11] and in extracts of yeast[12], while the other activity ('alkaline sucrase') differed from these invertases in optimal *p*H of action and its apparent specificity.

The enzyme extracts were prepared by homogenizing fresh plant material with water or 0·01 *M* phosphate buffer, *p*H 7·2–7·5 (1/3–1/2 vol. for embryos and 3 vol. for cotyledons), centrifuging at 34,000*g* for 30 min. at 0° C. and dialysing the supernatant solution against 0·01 *M* phosphate buffer, *p*H 7·0. Usually for qualitative work an equal volume of enzyme extract was added to a volume of buffer 0·2 *M* containing 0·2 *M* substrate to give the required *p*H after mixing. The enzyme action was followed by conventional chromatographic techniques which would detect 1–2 per cent hydrolysis[13] of the substrates. For quantitative work the reducing sugars produced were estimated by the Nelson–Somogyi method[14] after removal of the protein from the extracts by precipitation with zinc sulphate–barium hydroxide[15].

The 'alkaline sucrase' activity could be found in extracts prepared from embryos and cotyledons of ungerminated and germinated seeds but could best be prepared from embryos of steeped seeds. On 0·1 *M* buffered sucrose solutions at 25° C., the activity was found to have a narrow optimal *p*H range with a maximum at *p*H 7·8. The activity was not lost on dialysis and phosphate ions were not necessary for its action since it functioned equally well on sucrose in borate buffer (0·1 *M*); however, it was inhibited by *tris*(hydroxymethyl)aminomethane (*tris*)-hydrochloric acid and *tris*-maleate buffers. An extract of 'alkaline sucrase' prepared as described from steeped embryos had an initial rate of inversion of sucrose of 3×10^{-4} moles per hr. on 0·1 *M* sucrose, *p*H 7·8 at 25° C., which fell off to a low rate after 24 hr. of reaction and 40–50 per cent inversion of the sucrose. The extract showed no action towards stachyose, raffinose, 1-kestose, and, moreover, showed no transferase activity[13] during its action on strong (20 per cent) sucrose solutions. A small amount of hydrolysis observed in the experiments with stachyose and raffinose was shown to be due to the action of the α-galactosidase. This produced some sucrose which was then hydrolysed by the 'alkaline sucrase'. No manninotriose or melibiose were found in these experiments. These oligosaccharides would be found if invertase were present (as in the experiments with germinated embryos). The extract had some action on maltose at *p*H 7·8 but had no action on melezitose. The rate of hydrolysis of the maltose was 0·41 the rate of hydrolysis of sucrose by this extract under similar conditions. However, after purification by an ammonium sulphate fractionation, the rate of maltose hydrolysis was 0·2 of the rate of hydrolysis of sucrose. This separation indicated that two different enzymes were present, namely, sucrase and maltase, since if only one enzyme were involved the ratio of rates of hydrolysis on the two substrates would remain the same after purification.

On the other hand, the sucrase similar to other invertases could only be found in extracts prepared from germinated embryos (cf. Pridham[8]). This activity had a broader optimal *p*H range with a maximum at *p*H 4·9–5·0. An extract, prepared as described from germinated embryos, had an initial rate of inversion of sucrose approximately the same as that of the 'alkaline sucrase' extract prepared from steeped embryos. However, the rate of action was linear with time up to 90 per cent inversion of 0·1 *M* sucrose and the sucrose was completely inverted after 12–14 hr. The extract hydrolysed stachyose and raffinose, producing manninotriose and fructose, and melibiose and fructose respectively; however, the α-galactosidase activity which was also present in the extract hydrolysed the oligosaccharides further to give galactose, glucose and fructose as the final hydrolysis products. The extract also hydrolysed 1-kestose, maltose and melezitose and showed characteristic transferase action on strong (20 per cent) sucrose solutions. The hydrolysis of raffinose and melezitose would indicate that both β-fructosido—and α-glucosido—invertases were present in the extract.

From these facts it seems, therefore, that the new enzyme 'alkaline sucrase' is not: (*a*) an α-glucosido- or a β-fructosido-invertase since it does not attack melezitose and raffinose, which are respective substrates for these enzymes; (*b*) an α-glucosidase proper since the enzyme does not attack melezitose and maltose; or (*c*) sucrose phosphorylase because the enzyme hydrolysed sucrose in the absence of phosphate ions. It is concluded that the observed activity must result from the presence of a new type of sucrase, apparently highly specific for sucrose. The metabolic significance of this enzyme is not yet known.

A sucrose-specific enzyme has been found in a haploid hybrid *Saccharomyces* strain by Hestrin and Lindegren[16], and it is also interesting to note that two phytases having different *p*H optima and two lipases having different *p*H optima have been reported to occur in germinating lettuce seeds[17].

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[1] French, D., *Adv. in Carbohyd. Chem.*, **9**, 149 (1954).
[2] Dupéron, R., *C.R. Acad. Sci., Paris*, **241**, 1817 (1955).
[3] Dupéron, R., *Rev. gén. Botan.*, **61**, 261 (1954).
[4] Courtois, J. E., Anagnostopoulos, C., and Petek, C., *C.R. Acad. Sci., Paris*, **238**, 2020 (1954).
[5] Courtois, J. E., and Petek, F., *Bull. Soc. Chim. Biol.*, **36**, 1115 (1954).
[6] Courtois, J. E., Anagnostopoulos, C., and Petek, F., *Enzymologia*, **17**, 69 (1954).
[7] Courtois, J. E., Anagnostopoulos, C., and Petek, F., *Congr. intern. biochim. Résumés communs. 2e Congr., Paris*, 240 (1952).
[8] Pridham, J. B., *Nature*, **182**, 1687 (1958).
[9] Brill, H. C., *Philippines J. Sci.*, **12**, A, 1 (1917).
[10] Courtois, J. E., Anagnostopoulos, C., and Petek, F., *Bull. Soc. Chim. Biol.*, **35**, 73 (1953).
[11] Hattori, S., and Shiroya, T., *Arch. Biochem. Biophys.*, **34**, 121 (1951).
[12] Sumner, J. B., and Myrback, H. (eds.), *The Enzymes* (Academic Press, Inc., N.Y., 1951).
[13] Bacon, J. S. D., and Edelman, J., *Biochem. J.*, **49**, 529 (1951).
[14] Nelson, N., *J. Biol. Chem.*, **153**, 375 (1944).
[15] Somogyi, M., *J. Biol. Chem.*, **160**, 69 (1945).
[16] Hestrin, S. S., and Lindegren, C. C., *Nature*, **165**, 158 (1950).
[17] Mayer, A. M., *Enzymologia*, **19**, 1 (1958).

PHYSIOLOGY

Active Cation Transport as a Pace-maker of Respiration

INHIBITION of metabolism leads to a gain of sodium and a loss of potassium by cells, and it is widely realized that the maintenance of concentration gradients of these ions between cells and plasma depends on a supply of energy from metabolism. This holds true for red cells that derive energy from glycolysis, for the majority of tissues that depend on respiration and for the few cells and tissues that actively transport cations with energy derived from either metabolic pathway. Cells appear to transport cations actively only when adenosine triphosphate (ATP) is being produced *in situ* or is supplied from an exogenous source, such as by injection into poisoned squid giant axons[1] or by introduction into red cells ghosts by the technique of 'reversible hæmolysis'[2]. ATP is necessary, therefore, and its rate of production either by substrate or oxidative phosphorylation is one of the factors that determines the rate at which active transport occurs.

The converse situation is that the rate of metabolism might be regulated by the rate of active transport. This is feasible in respiring cells because respiration is governed by the concentration of adenosine diphosphate (ADP) which is determined in turn by the activity of the various mechanisms causing the hydrolysis of ATP. Since one of these is the fission of ATP due to active transport, it is of interest to test experimentally if the cessation of active transport by specific inhibitors which do not directly inhibit respiration causes a fall in the rate of respiration.

Slices of brain and kidney cortex from rabbits are suitable experimental tissues, because they have a high consumption of oxygen, on which active cation transport depends[3], and also the rates of exchange of their sodium and potassium are high. The maintenance of the intra-cellular potassium concentrations has therefore been compared with the Qo_2 (μl./mgm. dry wt./hr.) after incubations of 1 hr. in a saline medium containing glucose (150 m*M* sodium chloride, 5 m*M* potassium chloride, 2 m*M* magnesium chloride, 10 m*M* sodium phosphate *p*H 7·4, 10 m*M* glucose). Two ways of abolishing active transport were tested at 37° C.: (1) exposure of the slices to the cardiac glycoside, ouabain; (2) replacement of sodium of the medium with choline. With brain slices, 5 μ*M* ouabain caused falls of 70 per cent in the potassium concentration (from 360 to 100 m. equiv./kgm. dry wt.) and of 50 per cent in the Qo_2 (from 16·7 to 8·0). Replacement of sodium of the medium with choline caused a fall in Qo_2 to 8·8 and a fall in potassium concentration to 110 m.equiv./kgm. dry wt.; no further fall occurred with 5 μ*M* ouabain. Similar results were obtained with kidney slices, in which 25 μ*M* ouabain caused a fall of 64 per cent in the potassium concentration (from 420 to 150 m.equiv./kgm. dry wt.) and of 45 per cent in the Qo_2 (from 13·2 to 7·2). In a choline (sodium-free) medium, the Qo_2 of 8·4 and the potassium concentration of 120 m.equiv./kgm. dry wt. were unchanged by ouabain. The fall in Qo_2 caused by ouabain thus occurred only in the presence of sodium in the medium and is of the same magnitude as the reduction caused by the removal of sodium.

To see if the component of the tissue which is sensitive to ouabain and sodium still controlled respiration after breaking up the cells, homogenates of brain cortex were made in four solutions so as to introduce (0·15 *M* sodium chloride) or to exclude (0·15 *M* potassium chloride, 0·15 *M* choline chloride and 0·25 *M* sucrose) sodium. Each solution also contained 2 m*M* magnesium chloride, 10 m*M* glucose and 2·5 m*M* dipotassium hydrogen phosphate. Incubations were then made with and without ouabain (5 μ*M*) both in the absence of sodium and after the addition of sodium to part of the sodium-free homogenates. Table 1 shows that, irrespective of the method of preparation, homogenates in a high-sodium medium were sensitive to ouabain, the Qo_2 being reduced by 32–48 per cent (line *c*). Omission of sodium from the medium caused falls in Qo_2 of 33–42 per cent (line *g*) and, in the absence of sodium, no further fall in Qo_2 was caused by ouabain (line *f*). The respiration of homogenates was thus equally reduced by ouabain and the deprivation of sodium in a way comparable to that of slices.

Since systems utilizing ATP are some of the most important regulators of cell respiration[4], it seems likely that the reduction in Qo_2 is due to the sensitivity to ouabain and sodium of one or more of the reactions leading to the formation of ADP from ATP. Recent work on adenosine triphosphatases in red cells[5], kidney[6], brain[7] and crab nerve[8] has indeed shown the existence of ATP-hydrolysing systems which require sodium for activation and which are inhibited by ouabain, and it has been suggested that they are intimately associated with active transport. The quantitative relation between the amount of ATP split and the amount of material actively transported is difficult to assess because of insufficient information of the intracellular concentrations of ATP, ADP and orthophosphate, and estimates based on the standard free energy of hydrolysis of ATP are dubious[9]. Nevertheless, the results with slices show that the maintenance of a high tissue-potassium concentration and the Qo_2 are reduced in a parallel manner by ouabain and the deprivation of sodium, suggesting a partial control of respiration by the transport mechanism. The stoppage of active transport reduces the demand for ATP and therefore explains the concomitant fall in Qo_2 of 40–50 per cent. Similar results with homogenates suggest the possibility either that other ATP-hydrolysing reactions are also sensitive to ouabain and sodium or that the transport mechanism continues to hydrolyse ATP in broken cells. The latter possibility would seem more likely in view of the correlation of the properties

Table 1. DECREASE IN Qo_2 OF BRAIN HOMOGENATE IN THE PRESENCE OF OUABAIN AND ON THE OMISSION OF SODIUM FROM THE MEDIUM. (*S.E.* of the means are given)

Main solute in the solution in which homogenate made			Qo_2			
			Sodium chloride	Potassium chloride	Choline chloride	Sucrose
No. of experiments			3	5	4	4
High sodium medium (112–150 m*M* sodium)	no ouabain	(*a*)	2·70	2·62 ± 0·23	2·33 ± 0·19	1·65 ± 0·11
	with ouabain	(*b*)	1·40	1·69 ± 0·07	1·59 ± 0·14	1·09 ± 0·06
	fall due to ouabain	(*c*) = (*a* − *b*)	1·30	0·93 ± 0·23	0·74 ± 0·18	0·56 ± 0·08
Sodium-free medium	no ouabain	(*d*)	—	1·57 ± 0·14	1·49 ± 0·10	1·11 ± 0·10
	with ouabain	(*e*)	—	1·51 ± 0·11	1·43 ± 0·07	1·00 ± 0·06
	fall due to ouabain	(*f*) = (*d* − *e*)	—	0·06 ± 0·10	0·06 ± 0·09	0·11 ± 0·05
Fall due to omission of sodium from medium	no ouabain	(*g*) = (*a* − *d*)	—	1·05 ± 0·32	0·84 ± 0·20	0·54 ± 0·08

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of the active cation transport system in intact red cells with those of the ATP-hydrolysing system in fragment red-cell membranes[5].

The parallelism of the effects of ouabain and sodium on the maintenance of the tissue-potassium concentration and on the Qo_2 therefore suggests the tentative conclusion that an ATP-hydrolysing system associated with the active transport of cations may act as a pace-maker for about half the respiration of brain and kidney cortex.

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[1] Caldwell, P. C., Hodgkin, A. L., Keynes, R. D., and Shaw, T. I. *J. Physiol.*, **152**, 561 (1960).
[2] Gardos, G., *Acta Physiol. Acad. Sci. Hung.*, **6**, 191 (1954). Whittam, R., Ph.D. thesis, Univ. Cambridge (1957). Hoffman, J. F., *Fed. Proc.*, **19**, 127 (1960).
[3] Mudge, G. H., *Amer. J. Physiol.*, **165**, 113 (1951). Terner, C., Eggleston, L. V., and Krebs, H. A., *Biochem. J.*, **47**, 139 (1949). Pappius, H. A., and Elliott, K. A. C., *Canad. J. Biochem. Physiol.*, **34**, 1053 (1956).
[4] Krebs, H. A., and Kornberg, H. L., *Ergebn. Physiol.*, **49**, 212 (1957). Lardy, H. A., and Wellman, H., *J. Biol. Chem.*, **195**, 215 (1952). Siekevitz, P., and Potter, V. R., *J. Biol. Chem.*, **201**, 1 (1953). Chance, B., and Williams, G. R., *J. Biol. Chem.*, **217**, 383 (1955).
[5] Post, R. L., Merritt, C. R., Kinsolving, C. R., and Albright, C. D., *J. Biol. Chem.*, **235**, 1796 (1960). Dunham, E. T., and Glynn, I. M., *J. Physiol.*, **156**, 274 (1961).
[6] Wheeler, K. P., and Whittam, R. (unpublished results).
[7] Hess, H., and Pope, A., *Fed. Proc.*, **16**, 196 (1957). Jarnefelt, J., *Biochim. Biophys. Acta*, **48**, 104 (1961).
[8] Skou, J. C., *Biochim. Biophys. Acta*, **42**, 6 (1960).
[9] Whittam, R., and Davies, R. E., *Nature*, **173**, 494 (1954).

Effect of Thyroxine and 2,4-Dinitrophenol on the Rate of Utilization of Glucose

It has long been recognized that 2,4-dinitrophenol increases oxidative metabolism. The increase of oxygen consumption after the addition of 2,4-dinitrophenol (DNP) is explained as being a result of dissociation between oxidative and phosphorylative processes. Similarly, thyroxine accelerates oxidation by uncoupling an oxidative phosphorylation. But DNP does not prevent the phenomena of hypothyroidism other than basal metabolism[1], and fails to change lipid metabolism[1,2] and nitrogen excretion, the effects of which are known to follow thyroxine administration[1]. Therefore, it was interesting to compare the effects of these agents on carbohydrate metabolism.

In the present experiments I studied the influences of DNP and thyroxine on the rate of utilization of glucose in normal and thyroidectomized rabbits (120 days after operation). The utilization of glucose was determined from the rapid intravenous glucose tolerance test (GTT) which was performed 4 hr. following the subcutaneous administration of DNP (7·5 mgm. rabbit) or thyroxine (0·25 mgm./kgm. body-weight). Immediately after a fasting blood sample of venous blood had been obtained from the vein of the ear, 0·5 gm./kgm. body-weight of glucose was injected intravenously during 10 ± 5 sec. The blood samples for estimation of blood glucose were drawn from the vein of the other ear at intervals of 10, 20, 30, 45, 60, 75, 90 min. after the end of the injection of sugar. From this intravenous GTT the rate of utilization of glucose may be expressed by determining the assimilation coefficient of glucose (K) as was described in previous papers[3,4].

The results of my experiments are summarized in Table 1. 4 hr. after the administration of thyroxine and DNP the fasting blood-sugar levels were slightly increased both in normal and thyroidectomized rabbits. In intravenous GTT it was observed that thyroxine accelerated the utilization of glucose in both groups of experimental animals as early as 4 hr. after administration. Similarly, DNP increases the rate of utilization of glucose in normal and thyroidectomized rabbits. The results of these observations suggest that the influence of thyroxine and DNP on carbohydrate metabolism is similar, in contrast to their effects on lipid or protein metabolism. Both agents accelerate the rate of utilization of glucose, but the precise mechanism of this action is not clear. The increase in the assimilation coefficient of glucose after the addition of DNP may be explained by insulin mobilization (probably due to its hyperglycæmic effect), as suggested by Heténi *et al.*[5], or by the acceleration of the transfer of sugar across the muscle cell-membrane as observed by Randle *et al.*[6,7]. Further, it is interesting to note that thyroxine accelerates the utilization of glucose as early as 4 hr. following its injection, without any protracted latent period, which is in agreement with the experiments of Christophe and Mayer performed on rats[8].

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[1] Sollmann, T. (edit. by), *A Manual of Pharmacology*, 7 (W. B. Saunders, New York, 1948).
[2] Castor, C. W., and Beinwaltes, W., *J. Clin. Endocrinol.*, **10**, 1026 (1950).
[3] Macho, L., *Acta Med. Scand.*, **160**, 477 (1958).
[4] Macho, L., *Arch. Internat. Physiol. Biochem.*, **66**, 260 (1958).
[5] Heténi, jun., G., Issekutz, jun., B., and Winter, M., *Acta Physiol. Acad. Sci. (Hung.)*, **7**, 287 (1955).
[6] Randle, P. J., and Smith, G. H., *Biochem. J.*, **70**, 490 (1958).
[7] Morgan, H. E., Randle, P. J., and Regen, D. M., *Biochem. J.*, **73**, 573 (1959).
[8] Christophe, J., and Mayer, J., *Endocrinol.*, **65**, 475 (1959).

Determination of Spontaneous Plasma Fibrinolysis by a Practical Procedure

In a recent review I analysed present aspects and principles of the various methods that are generally used for assessing the phenomenon of proteolysis in the blood. The extensive significance of *in vitro* determination of whole plasma spontaneous fibrinolysis, which expresses a more faithful and exact picture of the evolution of the fibrinolytic mechanism in the circulating blood, was emphasized[1].

As is known, the determination of the spontaneous fibrinolysis of the whole blood or of plasma in physiological conditions takes a long time[2,3]. Repeated attempts to shorten the time of lysis by a strong dilution of plasma[4] or the carrying out of the low-

Table 1. Values of Fasting Blood-Sugar Levels (G) and of Assimilation Coefficient of Glucose (K) in Rabbits before (G_1,K_1) and after Thyroxine (G_2,K_2) or DNP (G_3,K_3) Administration

	No.	G_1	K_1	K_2	G_2	K_3	G_3
Normal rabbits	6	130 ± 3	1·08 ± 0·04	1·34 ± 0·07*	145 ± 10	1·52 ± 0·19*	151 ± 3†
Thyroidectomized rabbits	5	115 ± 7	0·86 ± 0·03	1·33 ± 0·08†	139 ± 16	1·63 ± 0·13†	126 ± 8

Statistical significance: * = $P < 0.05$; † = $P < 0.01$.

temperature technique[5] did not solve the problem sufficiently in so far as to establish an accepted practical method for the assessment and computation of the phenomenon of fibrinolysis. The only useful determination is obtained with the euglobulin method in the plasma, which according to different techniques that are described, offers an estimate of the phenomenon within 3–24 hr.[6–9]. This method is characterized by an expression for the spontaneous fibrinolysis of the plasma, but there are theoretical and practical considerations on the basis of which it becomes questionable if, and to what extent, the method is representative.

In this communication a practical procedure is described for the determination of spontaneous fibrinolysis in the whole plasma, based on the observation that the time of lysis of plasma under similar conditions of dilution, *p*H, recalcification and temperature is markedly shortened, if at first the received whole blood is diluted immediately in an appropriate buffer solution and, thereafter, the fibrin-clot is formed by recalcification of the supernatant fluid phase. Under these conditions the retraction of the clot is insignificant and thus the end-point of lysis can be observed.

Reagents :

(1) Buffer diethylbarbiturate (*p*H 7·40) :	
Sodium diethylbarbiturate	4·12 gm.
M/10 hydrochloric acid	144·00 ml.
Potassium oxalate	0·50 gm.
Sodium chloride	5·50 gm.
Water distilled at	1000·00 ml.
(2) *M*/600 calcium chloride	

With a 1·0 ml. calibrated precision-syringe, 0·5 ml. of venous blood is withdrawn and transferred immediately into a small tube containing 2 ml. of the buffer, which previously has been placed in a container of melting ice. The transfer is performed gently, while the mixing takes place automatically. The tube is centrifuged for 1 min. at 3,500 r.p.m. From the supercooled layer 1·0 ml. is taken at once and placed in a tube which is immersed in a 37° C. water-bath containing 1·0 ml. from the solution of *M*/600 calcium chloride. Clotting takes place in a few minutes. Time of lysis is determined by frequent checking.

The time of clot lysis varied between 6 and 18 hr. for almost the total series of cases, as this is calculated from the samples that were taken at the ninth hour, from 50 normal subjects of both sexes, 20–45 years of age (Fig. 1). In two cases a time less than 6 hr. (3 and 5 hr. respectively) was observed while for only 4 other cases the time of lysis was more than 18 hr. (21, 26, 27 and 32 hr.). The repeated trial of the method is satisfactory, because the different time of clot-lysis obtained in repeated determinations of the same plasma did not exceed 10 per cent.

The time-values obtained are much lower than with any hitherto known method of determination in whole plasma and comparable to the euglobulin method. The euglobulin method is used by many research workers as a method for the determination of the spontaneous fibrinolytic activity of the plasma, and this cannot be ascertained by virtue of the nature of the method, because lysis is only countable in the euglobulin fraction. These observations are of special import, particularly after recent work[6] showing that findings on the same samples of blood that are registered in the whole plasma and in the euglobulins fraction are not always in an analogous ratio. For example, examination of a hepatic patient, though it disclosed an increased fibrinolytic action in the whole plasma, did not record deviations worthwhile from the physiological point of view, by the method of the euglobulins.

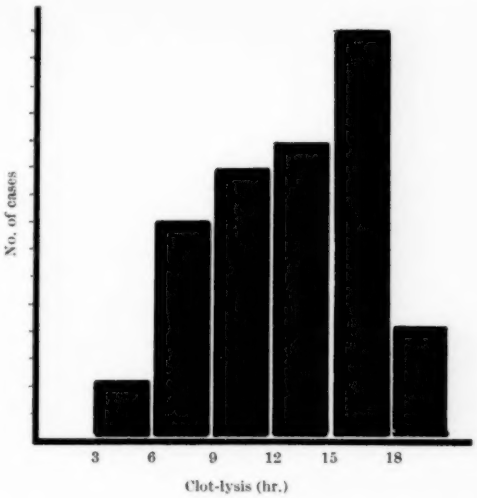

Fig. 1

The suggested procedure for measuring fibrinolytic activity of whole plasma represents a rapid technique and its simplicity is advantageous. This method requires 0·5 ml. of blood, the lysis of the fibrin clot is estimated accurately in a physiological *p*H and reproducibility is satisfactory.

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[1] Yatzidis, H., *Rev. Franc. Etudes Clin. et Biol.*, **4**, 383 (1959).
[2] Macfarlane, R. G., *Lancet*, i, 10 (1937).
[3] Ratnoff, O. D., *Bull. Johns Hopk. Hosp.*, **84**, 29 (1949).
[4] Biggs, R., Marfarlane, R. G., and Pilling, J., *Lancet*, i, 402 (1947).
[5] Fearnley, G. R., Balmforth, G., and Fearnley, E., *Clin. Sci.*, **16**, 645 (1957).
[6] Kowalski, E., Kopec, M., and Niewiarowski, S., *J. Clin. Path.*, **12**, 215 (1959).
[7] Milstone, H., *J. Immunology*, **42**, 100 (1941).
[8] Sherry, S., Lindemeyer, R. I., Fletcher, A. P., and Alkjaersic, N. *J. Clin. Inv.*, **38**, 810 (1959).
[9] Von Kaulla, K. N., *Circulation*, **17**, 187 (1958).

Effect of Bee Venom on Concentration of Blood Glucose and Liver Glycogen in Rabbits

Cobra venom and scorpion toxin are known to cause hyperglycæmia[1] whereas the *Walterinnesia* venum produces hypoglycæmia.

In the present experiments it was shown that bee venom, the effects of which resemble in many ways those of cobra venom, also causes hyperglycæmia and loss of liver glycogen. The blood glucose was determined by the Hagedorn and Jensen method[2]; the liver glycogen after hydrolysis of the removed livers by the method of Good, Kramer and Somogyi[3].

Fig. 1 illustrates the hyperglycæmia produced by a sub-lethal dose of 5 mgm. bee venom injected subcutaneously into a 2-kgm. fasting rabbit. The effect did not occur when the injections were made into immunized rabbits.

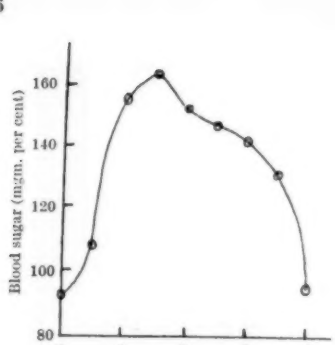

Fig. 1. Changes in blood-sugar level caused by injection of sub-lethal dose of bee venom

The fall in liver glycogen produced by 5 mgm. bee venom is illustrated for two groups of experiments. For each group five rabbits were used, one as a control, and the other four were killed 1, 2, 3 and 4 hr. after the injection and the liver removed.

Table 1. Effect of Sublethal Dose

Time after injection (hr.)	Control	Liver glycogen (mgm./100 gm.) (mean value)	
		Experiment 1	Experiment 2
0	164·66	166·33	159·66
1	164	153·66	143·33
2	162	131	121·33
3	164	129·66	119
4	159	119·66	118

Since the maximal fall in liver glycogen occurred during the second hour, mobilization of liver glycogen may fully account for the hyperglycæmia, which was maximal 1½ hr. after the injection. The effect may be a direct action of bee venom on the liver, or brought about indirectly by the release of adrenaline from the suprarenals, since Feldberg[4] has shown that bee venom as well as cobra venom injected into the cœliac artery of the cat produced a sustained release of adrenaline from the suprarenals.

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[1] Bertrand, M., and Vladesco, L. A., *C.R. Acad. Sci., Paris*, **209**, 585 (1939). Mohamed, A. H., *Nature*, **166**, 734 (1950).
[2] Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, **135**, 46 (1923).
[3] Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, **100**, 485 (1933).
[4] Feldberg, W., and Kellawan, C. H., *J. Physiol.*, **90**, 257 (1937).

Action of the Hypothalamus

Experiments with an analogue model of the heart, demonstrated by me[1], show that a pulse may arise, in a fluid-filled elastic system, in either of two ways: in the first, energy is cycled into the system from a mechanical source, corresponding to the physico-chemical contraction of the ventricles; in the second, pulsation can be derived from the static head of pressure with the aid of a length of elastic tubing arranged as a wave guide. The latter device, termed an 'oscillator', may be employed to time delivery of energy from the external source, in such a manner that the system is self-oscillating and that the two pulses, of differing origin, are synchronous.

In lesions of the conducting system of the heart, the coronary arterial system behaves, apparently, as an oscillator that utilizes the static head of pressure of the blood to develop a pulse. The same phenomenon is likely to occur at the circulus arteriosus, under normal conditions, however, due to the negative pressure in the internal jugular vein.

Examination of this possibility has led to the following explanation of the action of the hypothalamus:

Independently of the pulse from the heart, a vascular pulse originates in the perforating branches of the circulus arteriosus and is conveyed to the nerve cells in the nuclei of the hypothalamus. Provided the leading edge of the wave is sufficiently steep, the cells are excited to generate electrical impulses, which are utilized by the brain as timing signals.

There are at least two types of excitation. One, in the anterior hypothalamus, is associated with a positive temperature coefficient of frequency: a second, in the posterior hypothalamus, with a negative temperature coefficient of frequency. A third type of excitation probably occurs in the intermediate group of nuclei, with frequency unrelated to temperature. Similar physical relationships are found in connexion with the vibration of piezo-electric crystals, where the direction of the slice, with relation to the axes of the crystal, determines the sign of the temperature coefficient of frequency.

The importance of the structures around the circulus arteriosus suggests that the latter is the most important site in the body at which self-oscillation occurs. The major function of the sinus caroticus is, presumably, that of monitoring the diastolic blood pressure, to ensure stable operating conditions for the circulus arteriosus. Slight variations of frequency will result from changes of venous pressure in the thorax, due to respiration. This effect can be displayed on the model.

To prevent interference between the waves produced by the heart and those generated at the circulus arteriosus, it is essential that there be a co-ordinating mechanism. On one hand it is well known that signals—or nerve impulses—travel from the hypothalamus to the heart by way of the autonomic nervous system. On the other, it would appear that the signals from the heart to the hypothalamus are actually the heart sounds, conducted by the bones of the thorax and vertebral column to the region of the hypophyseal fossa, where they exert a 'pulsactor effect', of which the significance is explained elsewhere[2], on the surrounding vascular structures.

Examples of systems in which two synchronizing signals require co-ordination are to be found in radar. In one example, the transmitter takes signals from the master timing signal generator and, in addition, synchronizing signals from the indicator of the receiver: the more stable system determines the frequency. Applying the analogy to the human body, the hypothalamus is identified as the master timing signal generator.

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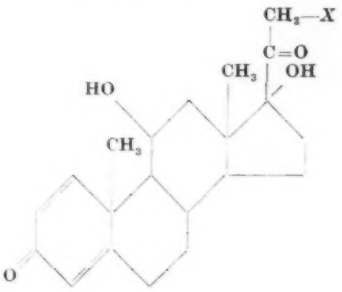
[1] Malcolm, J. E., *Proc. Physiol. Soc.* (March 24, 1961).
[2] Malcolm, J. E., *Nature*, **190**, 1114 (1961).

PHARMACOLOGY

Amino-derivatives of 11β,17α,21-Trihydroxy-3,20-Dioxo-1,4-Pregnadiene

THE skeleton of corticosteroids was systematically modified, mainly in order to study the changes in the pharmacological effect of the compounds.

One of the objects of our experiments was the exchange of the C_{21} hydroxyl group of corticosteroids by amino- and substituted amino-groups, respectively.



(*a*) $X = -O-SO_2-C_6H_4-CH_3$; (*b*) $X = -I$; (*c*) $X =$ -1 piperidyl; (*d*) $X =$ 4-morpholyl; (*e*) $X = N(C_2H_5)_2$.

The 21-tosylate (*a*) of 11β,17α,21-trihydroxy-3,20-dioxo-1,4-pregnadiene and the -21-iodine compound (*b*)[1] derived from it served as initial substances in preparing the compounds mentioned.

One mole of the -21-tosylate of 11β,17α,21-trihydroxy-3,20-dioxo-1,4-pregnadiene (*a*) was treated in a tetrahydrofuran solution, in a nitrogen current, with two moles of piperidine at 55° C. for 5 hr. On dropping the reaction mixture into water, 11β,17α,dihydroxy-3-20-dioxo-21(1-piperidyl)-1,4-pregnadiene (*c*) was obtained in 93 per cent yield, in crystalline form; on recrystallizing from a mixture of tetrahydrofuran and petroleum ether, melting point 181° C. (decomposition) was found. Analysis: calculated, C 73·06; H 8·6 per cent; found, C 73·00; H 8·9 per cent.

Compound (*c*) has also been prepared by reacting one mole of the corresponding iodine compound (*b*) with two moles of piperidine under conditions similar to those mentioned. Melting point of product: 181° C. (decomposition). Melting point of the hydrochloride: 236–238° C. (decomposition). Analysis: calculated, C 67·31; H 8·1; Cl 7·6 per cent; found, C 67·07; H 8·1; Cl 7·8 per cent.

In a similar way, 11β,17α,-dihydroxy-3,20-dioxo-21-(4-morpholyl)-1,4-pregnadiene (*d*) was obtained from the tosylate (*a*) and iodine compound (*b*). Melting point 191–193° C. Analysis: calculated, C 69·91; H 8·1 per cent; found, C 69·62; H 8·2 per cent. Melting point of hydrochloride: 235–237° C. (decomposition). Analysis: calculated, C 64·65; H 7·7 per cent; found, C 64·38; H 7·6 per cent.

Similarly to the above-mentioned compounds, 11β,17α,-dihydroxy-3,20-dioxo-21-diethylamino-1,4-pregnadiene (*e*) has been prepared. Melting point of hydrochloride: 227° C. (decomposition). Analysis: calculated, C 66·44; H 8·41; Cl 7·86 per cent; found, C 66·20; H 8·28; Cl 7·61 per cent.

C_{21}-amino -derivatives similar to the corticosteroid derivatives mentioned have also been prepared in an essentially similar way by E. J. Agnello and G. D. Laubach[2].

The compounds prepared by the foregoing technique were subjected to pharmacological tests in the form of bases insoluble in water and of water-soluble hydrochlorides, respectively. Compounds of gluco-corticoid activity are known to possess the general feature of containing oxo-groups on their C_3 and C_{20} atoms, a hydroxyl or oxo-group on their C_{11} atom and a double bond between C_4 and C_5. Also, a common property of these compounds is the hydroxyl function on C_{21}. The α-positioned hydroxyl on C_{17} is absent in the case of corticosterone of similarly gluco-corticoid effect, although only moderately active. Thus, all known efficient substances actually bear an oxygen atom on C_{21}.

The compounds mentioned previously were examined by comparison with the effect of prednisolone. These investigations were carried out by two methods. The accumulation of liver glycogen was investigated on suprarenal gland ectomized rats, extracting the deposited glycogen with trichloracetic acid and determining glycogen by turbidimetry. The anti-phlogistic action of the compounds was examined by the method of rat foot œdema determination evolved by us[3]. Male rats were used in these tests.

The experimental results obtained by both methods disclosed activities of about 80 per cent in respect to prednisolone in the case of the C_{21} piperidine derivative (*c*), of the C_{21} morpholyl derivative (*d*) and of the hydrochlorides of these compounds. Further, it was found that, while on treating rats *per os* with prednisolone the activity of this substance was about 50 per cent of that observed in the case of subcutaneous injections, the water-soluble derivatives were resorbed in 90 per cent.

On the basis of our experiments we can thus state that the C_{21} amino-derivatives of prednisolone possess a gluco-corticoid activity corresponding to that of prednisolone itself. Accordingly, the function of C_{21} does not seem to be an indispensable pre-requisite of gluco-corticoid activity. It appears that its function can be replaced by an amino-group. The water-soluble salts of the compounds prepared in this way are similarly efficient. From the gastro-intestinal tract these latter compounds are more readily resorbed than prednisolone—a substance insoluble in water.

Our experimental results will shortly be published in detail.

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[1] Nussbaum, A. L. (Schering Corp.), U.S. Patent 2814632 (Nov. 20, 1957).
[2] Agnello, E. J., and Laubach, G. D., U.S. Patent 2920999.
[3] Szporny, L., and Fekete, Gy., *Arch. Exp. Path. Pharm.*, **238**, 233 (1960).

Effects of Morphine-like Drugs in Chicks

SEVERAL workers[1–4] have used conscious chicks to study the actions of drugs. Chicks are particularly useful for drugs acting on the central nervous system, as their blood-brain barrier does not form until they are about 3 months old[5,6]. The effects of analgesic drugs on chicks were demonstrated at a meeting of the British Pharmacological Society[7] and are described

here. Analgesic action was measured by the multiple toe-pinch method[3], which has been applied successfully to guinea pigs and rats. An untreated chick squeaks and shakes its foot when an artery clip is applied to one of its toes and these responses were used to test whether nociception was suppressed by drugs.

Morphine showed very low potency in suppressing these responses in chicks, in contrast with its potency in other species. While morphine (5–20 mgm./kgm.), subcutaneously or intraperitoneally, suppresses comparable responses in mice, rats and guinea pigs, 200 mgm./kgm. intravenously was required to produce similar analgesic action in chicks. This dose was very close to the *LD*50 and the therapeutic index was remarkably low. Table 1 shows the relatively low therapeutic indices (*LD*50/*ED*50) of the analgesic drugs tested and shows that morphine, methadone and 1-(β-diethylaminoethyl)-2-(*p*-ethoxybenzyl)-5-nitrobenzimidazole (20684) have a far lower potency in chicks than in guinea pigs. Codeine, however, is about equipotent in the two species.

Table 1. The Analgesic Actions and Toxicities of Morphine-like Drugs in Chicks and a Comparison with their Analgesic Potencies in Guinea Pigs (ref. 8)

Compound	Chicks			Guinea pigs
	Analgesic *ED*50 (mgm./kgm.) intravenous	*LD*50 (mgm./kgm.) intravenous	$\frac{LD50}{ED50}$	Analgesic *ED*50 (mgm./kgm.) subcutaneous
Morphine	230	305	1·33	6·3
Methadone	>18	24	<1·33	3·0
Pethidine	>40	42	<1·07	20·4
Codeine	26	45	1·73	25·0
20684	3·2	>20	>6·25	0·0032

During this work, it was observed that the intravenous administration of morphine-like drugs quickly produced characteristic postural and behavioural effects in the chicks. These effects occurred at doses lower than those required to suppress responses to noxious stimuli. The chick exhibited marked ptosis, became restless and stiff-legged. Then it sat down with its feet spread apart and its back at 30–50° to the horizontal (Fig. 1). Some chicks whined occasionally and the intensity of their cries became stronger on provocation. These effects sometimes persisted for several hours but could be antagonized within a few minutes by nalorphine (50–100 mgm./kgm.), given subcutaneously or intravenously. Table 2 shows the approximate doses of analgesic drugs producing these characteristic and postural effects. Among many drugs of other types investigated, only atropine produced comparable postural effects.

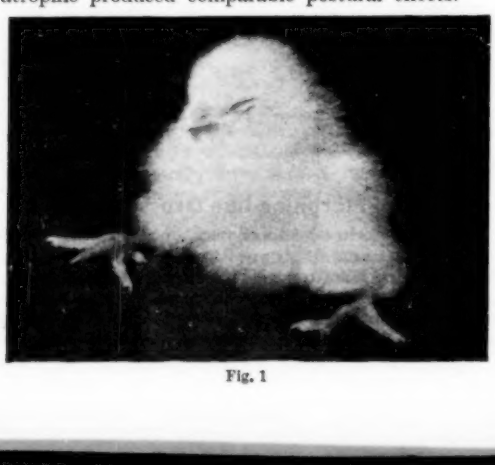

Fig. 1

Table 2. Approximate Minimal Doses of Analgesic Drugs producing Characteristic Postural Effects in Chicks

Compound	Dose (mgm./kgm.) intravenous
Morphine	20·0
Methadone	5·0–10·0
Pethidine	10·0
Codeine	15·0–20·0
20684	0·05

In conclusion, morphine and related drugs show only slight anti-nociceptive properties against acute mechanical stimuli in chicks, but cause postural and behavioural effects at low doses. Thus, in this species the side-effects of these drugs are more apparent than their anti-nociceptive action. The postural and other effects described appear to be characteristic of morphine and related drugs.

I thank Dr. H. O. J. Collier for his advice and encouragement, Dr. B. T. Warner for statistical advice and Ciba, Ltd., Basle, Switzerland, for a supply of 1-(β-diethylaminoethyl)-2-(*p*-ethoxybenzyl)-5-nitrobenzimidazole.

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[1] Selle, R., *Science*, **91**, 95 (1940).
[2] Clymer, N. V., and Seifter, J., *J. Pharmacol.*, **89**, 149 (1947).
[3] Buttle, G. A. H., and Zaimis, E. J., *J. Pharm. Pharmacol.*, **1**, 901 (1949).
[4] Zaimis, E. J., *J. Physiol.* (Lond.), **152**, 11 P (1960).
[5] Waelsch, H., *Biochemistry of the Developing Nervous System*, 198 (Academic Press, New York, 1955).
[6] Key, B. J., and Marley, E., *J. Physiol.* (Lond.), **155**, 39 P (1961).
[7] Schneider, C., *Brit. J. Pharmacol.*, **16**, 4 (1961).
[8] Collier, H. O. J., and Warner, B. T., *Brit. J. Pharmacol.* (in the press).

HÆMATOLOGY

Hæmoglobin *F* and the Genetic Control of Protein Structure

The publication of a hypothesis concerning hæmoglobin synthesis presented recently in *Nature* by Drs. Zuelzer and Robinson[1] prompts me to comment briefly. Their article concerns the genetic control of the synthesis of the peptide chains of the human hæmoglobins: *A* (adult) = $\alpha_2\beta_2$, *F* (fœtal) = $\alpha_2\gamma_2$, and A_2 (the minor adult hæmoglobin) = $\alpha_2\delta_2$. The symbols α, β, γ, δ are used to denote the peptide chains of which the hæmoglobin molecules are composed and also the genes controlling their structure. The authors propose that "the production of γ-chains is under the control of the same pair of genes which determines the formation of β-chains, once the maturation of the fœtus has reached the point where adult hæmoglobin can be made". Again, in cases of thalassæmia, for example, where there seemed to be a block in the synthesis of β-chains due to a substitution in the chain, "the simplest possible assumption seemed to be that the character or site of the substitution in the β-chain itself determined the nature of the by-product, γ or δ, accumulating in consequence of a block in the metabolic pathway of β-chain synthesis".

Zuelzer and Robinson's hypothesis suggests that the γ- and the δ-peptide chains represent alternative pathways in a synthetic mechanism which in the normal adult results in the production of β-peptide chains. The implication stated is that the alternative

pathways to the γ- and δ-chains branch off from the main pathway at, respectively, earlier and later stages of assembly of the peptide chain. A peptide chain will be assembled under control of a β-gene to the stage where it contains n amino-acids, and then completed by the β-gene, if that is normal. However, if there is a mutational alteration in the β-gene (as in hæmoglobin *S*, refs. 2, 3, and 4), then a proportion of unfinished chains of length n will take the alternative pathway controlled by the γ-gene, provided the mutation in β occurs after the branch point.

The elegant work of Dintzis[5] has shown that the two types of peptide chain of rabbit hæmoglobin are made sequentially by stepwise addition beginning at the N-terminus of the chains. Bishop *et al.*[6] had previously indicated that growth of the chain begins at the N-terminus. The size of these growth steps is not clear, but it cannot be larger than a few amino-acids. The same situation presumably exists in man. In that case, and according to Zuelzer and Robinson[1], the peptide chain grows from the N-terminal valine residue until it has reached the nth amino-acid. Growth continues to completion and a normal β-chain results, if the β-gene is normal and the person is an adult. However, in the case of a sickle-cell homozygote, who is abnormal in both his β-genes, a large amount of fœtal hæmoglobin, that is, of γ-chain, is made. In this case[1], growth after the nth amino-acid has been added continues along the alternative pathway under γ-gene control to a finished γ-chain, which is a γ-chain normal in its amino-acid sequence. Here the hypothesis is in difficulties on two counts; first, since we know[4] that the sickle-cell abnormality of the β-gene alters amino-acid number 6 in the β-chain (glutamic acid to valine), the number n, which is supposedly the common sequence between β and γ, must be less than 6. This is a small proportion of the total of 146 amino-acids[7]; nearly 96 per cent of the β- and γ-genes must be separate and must be operating independently, according to Zuelzer and Robinson. Secondly, the N-termini of the β- and γ-peptide chains are reminiscent of one another in sequence, but by no means identical: β-chain = Val. His. Leu. Thr. Pro. Glu. Glu. Lys. Ser....; γ-chain = Gly. His. Phe. Thr. Glu. Glu. Asp. Lys. Ser. ... . Therefore, the number n, the common structural sequence, must be zero. In other words, the whole of the β- and the γ-genes are independent and separate, at least in so far as their function in producing separate and independent β- and γ-polypeptide chains is concerned, in contrast to Zuelzer and Robinson's hypothesis. The idea that the α-, β-, γ- and δ-chains are produced by four genes which are separate has been discussed by several investigators (summarized in ref. 8).

The δ-gene, according to Zuelzer and Robinson[1], takes over at a later stage in synthesis where m amino-acids are already in the chain ($m > n$). From our knowledge of the δ-peptide chain, m must be smaller than 9 amino-acid residues, since Stretton[9] has shown that the β- and δ-chains are probably similar in sequence up to position 9, where instead of a serine residue (β-chain) he finds a threonine. However, sickle-cell homozygotes produce only normal δ-chains and therefore m, the sequence of amino-acids common to β- and δ-chains, must be less than 6 residues. Therefore, at least 96 per cent of β- and δ-genes are separate genes, producing separately controlled peptide chains.

Although the basic concepts of Zuelzer and Robinson's hypothesis are untenable, at least in so far as they can be tested in the human hæmoglobins, it must be admitted that the presence of considerable quantities of fœtal hæmoglobin in their cases of thalassæmia minor is somewhat puzzling. Thalassæmia is a disease occurring in different forms, for which we have at least two hypotheses[10], but perhaps not yet the definitive explanations. However, it seems likely that most of the phenotypes found in the thalassæmias and other hæmoglobinopathies can be explained by the function and malfunctioning, and by the interaction, of the alleles of the α-, β-, γ- and δ-genes at present recognized. These genes are conceived as consisting of four separate genes controlling the synthesis of the four chemically recognizable hæmoglobin chains[8]. Where there is a drastic curtailment or block of the synthesis of one of the peptide chains, it seems to me more fruitful, and usually adequate, to consider consequent increases in the production of other peptide chains as a process of compensation; perhaps this is brought about by a change in the control mechanism which normally regulates the amounts of peptide chain made by each gene.

Note added in proof: The recent work of Schroeder, W. A., *et al.* (*Proc. U.S. Nat. Acad. Sci.*, **47**, 811; 1961, and unpublished experiments) on the amino-acid sequences of the γ-peptide chain throws more light on the degree of similarity between the structure of the β- and γ-chains. The differences between them are fairly well spread along their lengths, and therefore regions of similarity are likely to be short and also scattered. In particular, at the C-termini of the β- and γ-chains only the last two amino-acids are the same, -Tyr.His, with dissimilar sequences before these. Therefore, any similarity between the β- and γ-chain genes at the ends corresponding to the C-termini of the peptide chains would have to be confined to a negligibly small proportion of the length of the genes.

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[1] Zuelzer, W. W., and Robinson, A. R., *Nature*, **190**, 237 (1961).
[2] Neel, J. V., *Science*, **110**, 64 (1949).
[3] Pauling, L., Itano, H. A., Singer, S. J., and Wells, I. C., *Science*, **110**, 543 (1949).
[4] Hunt, J. A., and Ingram, V. M., *CIBA Symp. Biochem. Human Genetics*, 114 (1959).
[5] Dintzis, H. M., *Proc. Nat. Acad. Sci.*, **47**, 247 (1961).
[6] Bishop, J., Leaky, J., and Schweet, R., *Proc. U.S. Nat. Acad. Sci.*, **46**, 1030 (1960).
[7] Braunitzer, G., Hilschmann, N., Hilse, K., Liebold, B., and Muller R., *Z. physiol. Chem.*, **322**, 96 (1960), and unpublished work.
[8] Ingram, V. M., in *Genetics*, Macy Found. Symp., edit. by Sutton, H. Eldon (1960).
[9] Ingram, V. M., and Stretton, A. O. W., *Nature*, **190**, 1079 (1961).
[10] Ingram, V. M., and Stretton, A. O. W., *Nature*, **184**, 1903 (1959).

THE basic assumption of our concept[1] of a common metabolic pathway with different end-products depending on the nature or location of substitutions within the hæmoglobin molecule is not the direction in which the synthesis of the polypeptide chain may proceed, but the very premise itself—that the build-up occurs in a stepwise fashion. This is the all-important conclusion which we reached on purely deductive grounds on the basis of the observation that mutations apparently involving β-chain synthesis, for example, the thalassæmias observed by us, could selectively determine whether an excess of γ- or δ-chains is formed.

At the time when we formulated this hypothesis, the concept of a stepwise build-up was a rather radical departure from the then prevalent ideas on the mode of operation of genetic templates. The almost immediate verification of this, our basic premise, by the work of Bishop[2], and that of Dintzis[3], which Ingram quotes, would seem to strengthen rather than weaken our hypothesis. Since no information was available at the time concerning the direction of the build-up, we refrained from making any explicit assumptions on this point. It is true that one can read the implication into our communication that we were suggesting the build-up of the molecule towards, rather than away from, the N-terminal. We were considering the alternative, but had no basis for a choice. We were interested rather in describing our concept in the simplest possible terms, and did not wish to confuse the reader by an alternative which was equally likely but would not materially affect the validity of the basic concept.

We are in complete agreement with Ingram's reasoning but not with his corollary. Now that it is known that the stepwise build-up in the formation of the molecule is at the N-terminal at least for rabbit, and very likely for human, hæmoglobin, it is clear that the correct model must involve convergence toward a common pathway a relatively short distance from the N-terminal, rather than divergence from the common pathway at that same point. It becomes simply a matter of reversing direction now that the necessary information is at hand. It is just as easily conceivable that, given a substitution near the N-terminal, the first few amino-acids are combined into an alternate pattern (for example, γ-chain type), from which the rest of the chain can arise in a normal manner along the common pathway, as it is to assume the opposite type of process. To that extent the hypothesis can now be made explicit. Instead of the 'branching-off point', we must speak of a 'point of convergence'.

Alternate explanations regarding the control of synthesis of hæmoglobin *F* are not particularly satisfying, as Ingram admits. It is, however, necessary for genetic theory to satisfy not only the structural relationships within the molecule, which no doubt will be clarified one way or the other in the near future, but also a large body of other pertinent observations. The theory of separate γ-genes runs into difficulties with respect to three sets of acts which can be reconciled with our present hypothesis.

One concerns the observations on thalassæmia minor with the stigmata of a β-gene mutation and divergent behaviour with respect to hæmoglobins *F* and A_2 which formed the basis of our theory[4]. The second is the increasingly well-documented allelism of the high *F*-gene with known β-genes such as *S* and *C*. Evidence from another laboratory will be forthcoming shortly to the effect that the γ-chains of high *F* carriers are identical in structure with those of normal fœtal hæmoglobin and that the high *F* in these individuals is evenly distributed throughout the red-cell population. This seems to us grounds for treating γ-chains as mutant β-chains under the control of the β-gene.

The third point is the uneven distribution of fœtal hæmoglobin in the red-cell population in various genetic and acquired disorders, for example, sickle cell anæmia, thalassæmia minor and leukæmia[5,6]. The concentration of hæmoglobin *F* in a few, rather than in all, cells suggests metabolic influences not under direct genetic control.

We are not attempting to defend at all costs a hypothesis which is, after all, a mere heuristic principle; but we think it is somewhat radical and premature to discard it on the basis of interpreting a relatively minor and peripheral implication as the substance of the argument.

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[1] Zuelzer, W. W., and Robinson, A. R., *Nature*, **190**, 237 (1961).
[2] Bishop, J., Leaky, J., and Schweet, R., *Proc. U.S. Nat. Acad. Sci.*, **46**, 1030 (1960).
[3] Dintzis, H. H., *Proc. U.S. Nat. Acad. Sci.*, **47**, 247 (1961).
[4] Zuelzer, W. W., Robinson, A. R., and Booker, C. R., *Blood*, **17**, 393 (1961).
[5] Betke, K., *Folia Haematol.*, **76**, 293 (1959).
[6] Coehn, F., and Zuelzer, W. W., *Thirty-first Ann. Meeting, Soc. Ped. Res.* (to be published).

PATHOLOGY

Immunological Study of experimentally induced Extrahepatic Cholestasis

Cohen *et al.*[1] have shown, by means of an immuno-cyto-chemical-fluorescence method, that gamma-globulin is present in the reticulo-endothelial cells of the sinusoids and fibrous tracts of human livers affected by hepatitis of various types and by post-necrotic cirrhosis. To test, in some measure, the unitary concept of liver disease put forward by Shorter *et al.*[2], it was decided to investigate whether similar changes occurred in livers damaged experimentally by the primarily extrinsic method of ligation of the common bile duct.

Rabbits of either sex and no particular breed but of 2 kgm. weight were used for the experiments. Under intravenous nembutal (Abbott) anæsthesia the common bile duct was exposed, ligated near its middle at two points separated by approximately 1 cm. and the intervening part excised. Penicillin powder was dusted over the operation site and the edges of the abdominal wound. Four groups of six animals were so treated and to each group was added a further control animal, which was subjected to exposure of the common bile duct but without ligation and excision of the latter. There were no operation deaths, and, apart from a small stitch abscess in the skin of one animal, no sepsis occurred.

The four groups, including their controls, were killed respectively at 3, 7, 14 and 28 days after operation and with each group a normal animal was also killed. The animals were killed by the intravenous injection of overdoses of nembutal. They were dissected immediately and portions of the right lobe of the liver and of the spleen were removed, frozen rapidly in a dry ice/alcohol mixture at −70° C. and stored at −30° C. while waiting to be sectioned.

Sections 6μ-thick were cut in a cryostat, exposed to a current of warm air (45° C.) for about 10 sec. and then washed for 5 min. in each of three changes of saline which was phosphate-buffered to *p*H 7·0. Each section was then treated for 30 min. with fluorescein-labelled anti-rabbit globulin of the sheep (Sylvana Chem. Co., New Jersey), washed as before in phosphate-buffered saline and mounted in glycerin. Immunological specificity was controlled by the treatment of two further batches of section either with non-conjugated sheep anti-rabbit globulin (Sylvana

Chem. Co.) or with normal sheep globulin (Wellcome Research Lab.) for 30 min., and, later, after washing in phosphate-buffered saline, with fluorescein-labelled sheep anti-rabbit globulin as before. These sections were also mounted in glycerin.

The sections prepared by both methods were examined immediately by fluorescent microscopy. The results were recorded by the method adopted by Cohen *et al.*, that is, the cells containing gamma-globulin were counted in each of at least three high-power (×40 objective; ×6 eyepiece) fields and the average for one field expressed thus: + = 2–3 cells; ++ = 4–6 cells; +++ = 7–9 cells; and ++++ = 10 or more cells.

In addition, sections from two experimental and both control animals in each group were prepared as above and then stained with hæmatoxylin and eosin; these sections were examined by bright-field microscopy.

Bright-field examination of the livers of the experimental animals in the first group, killed three days after operation, revealed marked accumulation of bile pigment in the hepatic parenchymal cells and bile ductules. Together with this cholestasis there were numerous foci of liver-cell necrosis, generalized enlargement of Kupffer cells, slight histiocytic and fibroblastic reactions in all the portal tracts and patchy proliferation of bile ductules. These changes were unaccompanied by any abnormality of the lobular architecture. The changes found in the second, third and fourth groups, examined 7, 14 and 28 days respectively after operation, were similar in nature and degree, with the exception of the fibroblastic reaction, to those present in the first group. In the later groups the fibroblastic reaction was greater and had also produced short collagenous projections from the portal tracts into the adjacent damaged parenchyma though without significant disturbance of the lobular pattern. In no case was there histological evidence of infection.

Gamma-globulin was found in cells lining the hepatic sinusoids of all the experimental animals of each group, but none was present in the portal tracts or elsewhere in the livers of these animals and there was none at any site in the livers of the control animals. The number of cells containing gamma-globulin increased with increase of the duration of cholestasis. The results are summarized in Table 1. Intracellular gamma-globulin was present in similar amounts in the splenic red pulp of the experimental and control animals of all the groups.

The sinusoidal cells in which the gamma-globulin was found appeared, on histological grounds, to be Kupffer cells. In no cases in the present experiments have these cells shown morphological changes indicating transition to plasma-cells. Although it is impossible to assess whether these cells elaborated or merely took up the gamma-globulin, the latter seems to be more likely in view of the enlargement of the cells, their known phagocytic powers and the lack of histological changes suggesting transition to plasma-cells.

The significance of these findings is obscure. It must be mentioned, however, that Havens[3] has conjectured that some forms of liver disease may be initiated or perpetuated by the formation of antibodies to the products of liver-cell necrosis, but whether this is the explanation of the liver damage which follows the experimental induction of extra-hepatic cholestasis must await the results of further investigations. On the whole, in agreement with Cohen *et al.*, it seems doubtful that the increase in hepatic gamma-globulin found in some diseases of the liver indicates the presence of any immunological process.

Table 1. CELLS CONTAINING GAMMA-GLOBULIN IN THE LIVER IN EXPERIMENTALLY PRODUCED EXTRA-HEPATIC CHOLESTASIS

Time interval in days	Groups	No. of animals	Liver	
			Cells containing gamma-globulin in portal tracts	Cells containing gamma-globulin lining sinusoids
3	Animals with ligated bile ducts	6	0	+
	Control animals	2	0	0
7	Animals with ligated bile ducts	6	0	+ to ++
	Control animals	2	0	0
14	Animals with ligated bile ducts	6	0	+ to +++
	Control animals	2	0	0
28	Animals with ligated bile ducts	6	0	+++ to +++
	Control animals	2	0	0

Finally, it seems that these results do not oppose a unitary concept of liver disease as a whole, since a change found in a wide variety of diseases of the liver, namely, the appearance of gamma-globulin in reticulo-endothelial cells lining sinusoids, has also occurred in uncomplicated experimentally produced extra-hepatic cholestasis.

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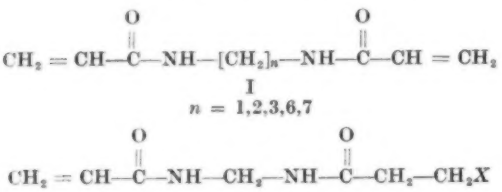
[1] Cohen, S., Ohta, G., Singer, E. J., and Popper, H., *J. Exp. Med.*, **111**, 285 (1960).
[2] Shorter, R. G., Paton, A., and Pinniger, J. L., *Quart. J. Med.*, **28**, 43 (1959).
[3] Havens, W. P., *Internat. Arch. Allergy and App. Immunol.* (Basle), **14**, 75 (1959).

N,N′-Alkylene-*bis*(Acrylamides), N-(Acrylamidomethyl)-3-Halopropionamides and Related Compounds: a New Series of Anti-tumour Agents

THE chemical, biological and clinical aspects of the 'alkylating agents' as anti-tumour agents have been reviewed thoroughly[1,2]. The available data indicate that at least two minimal requirements are necessary for possible alkylating agents[2]: (1) Such compounds must either have a certain chemical reactivity, or be converted *in vivo* to structures that do; (2) although polyfunctionality is not a rigid requirement for activity, the most active alkylating agents are polyfunctional. Functional groups with chemical reactivity are usually defined as those able to react with nucleophilic groups such as amino and mercapto, and the anions of carboxylic and phosphoric acids.

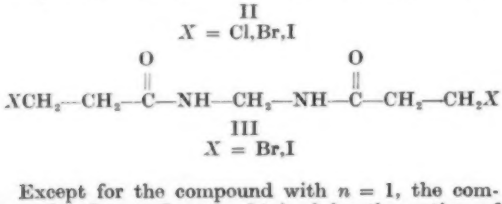
In the course of syntheses of compounds with potential anti-tumour activity, N,N′-methylene*bis*-acrylamide[3] [I, $n=1$] was submitted for screening and found to inhibit markedly the growth of two

transplanted mouse tumours (specific details later in this communication). Chemically, N,N′-methylene-*bis*acrylamide fulfils the requirements for an active alkylating agent, since both α,β-unsaturated linkages can easily add mercaptans or amines[4]. Inasmuch as this structure represented a new type of anti-tumour agent, an effort was undertaken to define more precisely the structural characteristics necessary for anti-tumour activity. As a result, compounds of three related though distinct structure types were found to be active against at least one transplanted mouse tumour. The general structures are as follows:



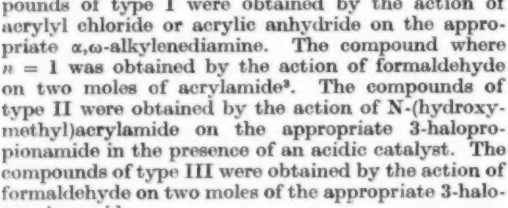
I

n = 1,2,3,6,7



II

X = Cl,Br,I



III

X = Br,I

Except for the compound with $n = 1$, the compounds of type I were obtained by the action of acrylyl chloride or acrylic anhydride on the appropriate α,ω-alkylenediamine. The compound where $n = 1$ was obtained by the action of formaldehyde on two moles of acrylamide[3]. The compounds of type II were obtained by the action of N-(hydroxymethyl)acrylamide on the appropriate 3-halopropionamide in the presence of an acidic catalyst. The compounds of type III were obtained by the action of formaldehyde on two moles of the appropriate 3-halopropionamide.

Compounds of type I wherein one or both acrylamide residues were replaced by methacrylamide (one) or β,γ-buteneamide residues (one and two) were also found to possess anti-tumour activity, while crotonamide, cinnamamide and sorbamide residues gave inactive compounds. The full chemical details on all the above compounds, as well as on numerous other inactive compounds, will be published elsewhere.

In our Laboratories, compounds are screened for anti-tumour activity against a spectrum of three transplanted tumours in *C3H* mice: sarcoma 180, 6*C3HED* lymphosarcoma, and *C3H* mammary adenocarcinoma. The details of the screening procedure have been published[5]. All the active compounds described before inhibited the growth of the *C3H* mammary adenocarcinoma. In addition, N,N′-methylene*bis*acrylamide inhibited the sarcoma 180, and N,N′-ethylene*bis*acrylamide [I, $n = 2$] inhibited the 6*C3HED* lymphosarcoma. One compound, N-(acrylamidomethyl)-3-bromopropionamide [II, X = Br], inhibited all three screening tumours. Evaluation studies showed that this compound was effective against all three tumours by oral administration as well as by intraperitoneal injection. It prolonged significantly the survival-time of mice bearing the transplanted *C3H* mammary adenocarcinoma, but was ineffective against 'spontaneous' mammary adenocarcinomas. In common with the alkylating agents, N-(acrylamidomethyl)-3-bromopropionamide acts as a bone marrow depressant[6]. Detailed results of the screening and evaluation studies will be published elsewhere.

N-(Acrylamidomethyl)-3-bromopropionamide is now undergoing limited preliminary clinical trials.

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[1] Schmidt, L. H., *Ann. N.Y. Acad. Sci.*, **68**, 657 (1958).
[2] Montgomery, J. A., *Cancer Res.*, **19**, 447 (1959).
[3] Lundberg, L. A., *U.S. Pat.* 2,475,846; *Chem. Abstr.*, **44**, 873 (1950).
[4] Dalton, P. B., *U.S. Patents* 2,576,501-2; *Chem. Abstr.*, **46**, 1809 (1952).
[5] Dearborn, E. H., *Acta Unio Intern. Contra Cancrum*, **15**, No. 1, 76 (1959).
[6] Vogel, A. W. (to be published).

Two Phycomycetes isolated from Subcutaneous Phycomycosis in Uganda

In 1956, Lie Kian Joe *et al.*[1] reported two cases of a curious subcutaneous granuloma in children in Indonesia. The tissues showed a characteristic histological picture in the midst of which lay fungus filaments with occasional septa. From one case they isolated many colonies of a phycomycete, *Basidiobolus*, not hitherto known to infect mammals. Later this group of workers has reported three more cases[2]; *Basidiobolus* was isolated from two of them and an unidentified phycomycete from the third. A sixth case, probably infected in Indonesia, has been recognized by biopsy examination in England[3].

The general term 'phycomycosis' was suggested for diseases caused by members of the Phycomyces[4] to avoid the restricted connotation of mucormycosis and to cover cases in which a phycomycete was demonstrated by histology or culture, but closer identification was not possible.

A chronic progressive subcutaneous granuloma affecting only children and producing a characteristic picture on biopsy examination has been recognized in Uganda for many years[5]. It is locally known as 'creeping granuloma' and, though never proved, it has been regarded as most likely due to some helminthic infection largely because of the small 'tunnels' in the sections and the heavy infiltration of eosinophil cells around them. Jelliffe *et al.*[6], however, concluded that their patient with 'creeping granuloma' was suffering from a mycotic infection which they recognized as the subcutaneous phycomycosis of Lie Kian Joe.

About the same time, and unaware of the previous work, I also had come to the conclusion from a study of a series of biopsies that 'creeping granuloma' was a mycotic infection, and was afterwards able to isolate *Basidiobolus ranarum* from the next case presenting with this condition. Prof. Lie has seen the biopsy from this patient and agrees that the picture is identical with that of the Indonesian cases. The identity of the 'creeping granuloma' of Uganda and the subcutaneous phycomycosis of Indonesia therefore seems clear.

In connexion with the isolation of *Basidiobolus* in Uganda (the first from human tissue outside Indonesia), it is of great interest to note that the report referred to in ref. 5 is not accurate where it says a fungus had been sought in one case without result. A fungus was isolated from this case and it was identified by the Mycological Reference Laboratory at the London School of Hygiene as a member of the *Entomophthora* group, but as no connexion was then known between any human disease and such a phycomycete, it was considered of no significance. The records at that Laboratory have been consulted and confirm that the fungus was a phycomycete of the order Entomophthorales, probably an *Entomophthora* but certainly not a *Basidiobolus*. The evidence both from Indonesia and Uganda thus suggests that subcutaneous phycomycosis may be caused by more than one kind of phycomycete.

There is as yet no evidence either from Indonesia or Uganda of the way in which a fungus such as *Basidiobolus* is transferred from its normal habitat in the intestinal contents of frogs and beetles to the subcutaneous tissue of children, and this is now being investigated.

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[1] Lie Kian Joe, Njo-Injo Tjoei Eng, Sutomo Tjokronegoro and Emmons, C. W., *Arch. Derm. Syph.*, **74**, 378 (1956).
[2] Lie Kian Joe and Njo-Injo Tjoei Eng, *Ann. N.Y. Acad. Sci.*, **89**, 4 (1960).
[3] Symmers, W. St. C., *Brit. Med. J.*, i, 1331 (1960).
[4] Lie Kian Joe, Njo-Injo Tjoei Eng, Sutomo Tjokronegoro, Schaafsma, S. J., and Emmons, C. W., *Amer. J. Clin. Path.*, **32**, 62 (1959).
[5] Proc. Mulago Staff Clin. Meeting, *E. Afr. Med. J.*, **33**, 365 (1956).
[6] Jelliffe, D. B., Burkitt, D. P., O'Connor, G. T., and Beaver, P. C., *J. Pediat.* (in the press).

BIOLOGY

Correlative Inhibition of Formation of Chlorophyll in the Leaves of Etiolated Pea Plants

Seventeen-day-old pea plants (*Pisum sativum* L., variety Přebohatý) grown in the dark were decapitated above the different nodes as illustrated by horizontal bars in Fig. 1. The decapitated plants and the isolated sections of the stems were placed in a window of the laboratory, left there overnight and on the next day exposed to daylight. After 24 hr. the chlorophyll formation in the individual organs was visually evaluated by means of a standard colour scale.

Fig. 1 shows that in intact control plants (*a*) the young leaves of the apex (*Aa*) and its region of elongation had become intensively green. The isolated stem tips (*Ab* ; *Af*) had formed chlorophyll to a lesser extent. In the decapitated plants chlorophyll formation took place mainly in the morphologically highest organ (*L2b* ; *L1c* ; *S2d*). Such organs and even the organs on isolated stem sections (*L2f* ; *L1f*) usually formed more chlorophyll than the corresponding organs on intact plants (*L2a*; *L1a*) or on stem sections with the stem tip intact (*L2d*; *L1d*). The experiment clearly shows that in leaves chlorophyll formation is inhibited even in the absence of the apex if there is a morphologically higher, that is, younger node present.

In the presence of the basal parts, on the contrary, chlorophyll formation (compare *S2d* with *S2f* or *Ae* with *Af*) is stimulated. Individual organs differ in their capability to form chlorophyll according to their age if the inhibiting apical parts are removed as may be seen by comparing *L2b*, *L1c*, *S2d* and *S1e*. The fact that inhibition in the presence of apical parts is expressed not only in the control of the growth of lateral buds but also in the control of chlorophyll formation in the leaves and scales stresses the necessity to look on the plant as a well-co-ordinated whole[1,2,6]. The present concept of apical dominance[6] emphasizes the importance of the stem tip controlling directly or indirectly the growth of lateral buds. If the totality of the plant is to be taken into consideration it seems necessary to extend this concept to the active function of the transport system[3–5,7] in the theory of correlations until now somewhat underestimated.

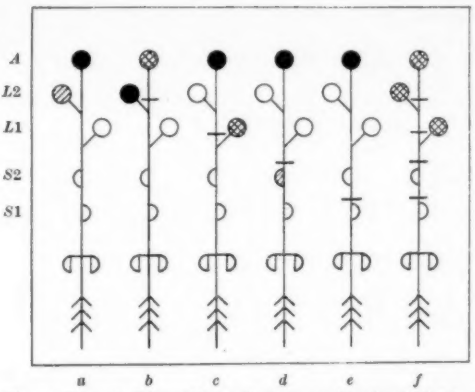

Fig. 1. Chlorophyll formation in the leaves and scales of etiolated plants and isolated stem sections of *Pisum sativum* after exposure to daylight for 1 day

In a normal etiolated pea plant it is the metabolic activity of the apex, driving the energy-requiring processes, that maintains the material, metabolic and electric gradients. These gradients in turn render possible a continuous flow of material to the apex, acting, with respect to this flow, as a metabolic sink or a centre of attraction. In such a process, positive[7] and negative[6] feedback mechanisms take part; auxins, gibberellins, kinetins and inhibitors together with their precursors represent probably important components of such systems.

If the transport, at least of some of the necessary material, by the conducting system is an active process, the 'pull of material'[5] by the conductive system continues even after the removal of the stem tip, so that one can speak about a 'memory' or 'inertia' of the system. This state probably has a more-or-less transient character; but it is supposed to play a decisive part in the first phases following the removal of the apex. Then, in analogy to a suddenly closed pipe line with an excessive pressure arising, the pulled material starts to flow to organs that were not reached before, partly due to resistances encountered.

The differences depending on age in chlorophyll formation by the leaves and scales are probably due to differences in such resistances of metabolic or mechanical character. It is assumed that similar relations participate in a variety of other phenomena studied by experimental morphology, such as the differences in mass production by the marginal buds of *Bryophyllum crenatum* leaves[2] or even the classical case of the cotyledonary bud inhibited by its cotyledon[2].

Apical dominance and similar phenomena are considered as special cases of metabolic correlations representing the basic principle of the totality of plant organism.

I wish to express my thanks to Prof. Rudolf Dostál for stimulating discussion.

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[1] Čaljachjan, M. Ch., *Celostnost organizma v rastitelnom mire* (Jerevan, 1955).
[2] Dostál, R., *O celistvosti rostlin* (Prague, 1959).
[3] Fensom, D. S., *Canad. J. Bot.*, **37**, 1003 (1959).
[4] Kursanov, A. L., Brovchenko, M. I., and Pariskaya, A. N., *Fiziologia Rastenii*, **6**, 527 (1959).
[5] Nelson, C. D., and Gorham, P. R., *Canad. J. Bot.*, **37**, 439 (1959).
[6] Scott, B. I. H., *Austral. J. Biol. Sci.*, **10**, 164 (1957).
[7] Spanner, D. C., *J. Exp. Bot.*, **9**, 332 (1958).
[8] Thimann, K. V., *Rapports et Communications 8e Congr. Intern. Bot.*, Sect. 11, 114 (Paris, 1954).

Flower Initiation in the Carnation in Response to Photoperiod

In 1932, Laurie and Poesch[1] reported that for glasshouse carnation an increase in photoperiod during the winter months led to earlier production of flowers in the spring. There have been a number of similar reports since, but views have differed as to whether photoperiod affects the time of flower initiation or only the rate of subsequent development of the flower. Post[2] and more recently Rünger[3] have claimed that only the latter stage of development is affected. Blake[4], however, working in the laboratories here, demonstrated an effect on flower initiation such that plants grown in short days of 8 hr. had more leaves below the flower than plants grown in long days where an additional 9 hr. of illumination was given. The object of the present communication is to report results from three glasshouse experiments which show unequivocally that time of flower initiation in carnation is affected by photoperiod and that this effect can occur independently of the total radiation received by the plant.

In experiments 1 and 2, plants were grown from normal shoot cuttings. These were cut back about one month after planting and one axillary shoot was allowed to develop. In experiment 3, plants were grown from 'leaf-bud cuttings' comprising an axillary bud, its subtending leaf and a small portion of stem. The variety White Sim was used throughout. The basic treatment was 8 hr. of natural daylight (short day). In the other two treatments this was supplemented by 2 hr. of light from 'daylight' fluorescent tubes plus tungsten filament lamps, given either as 1 hr. at either end of the day (extended day) or as 2 hr. at the middle of the dark period (interrupted night). In each of the three experiments sampling of the plants and dissection of their apices showed that flowers had been initiated in the 'interrupted night' treatment, while plants in the other treatments were still vegetative (Table 1).

Table 1

Experiment No.	Date of planting	Date of sampling	No. of plants with flower primordia out of six sampled		
			Short day	Extended day	Interrupted night
1	28.1.60	26. 5.60	None	None	5
2	15.6.60	12.10.60	None	None	6
3	23.4.60	6.10.60	None	None	3

Further treatments in the experiments quoted and further experiments now in progress here indicate that a number of factors can interact with photoperiod in effects on flower initiation. An understanding of these factors is desirable in considering any possible applications of photoperiodic treatments to the growing of carnations for cut flowers.

We wish to express our appreciation to the Agricultural Research Council, which has made a grant to support this work.

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[1] Laurie, A., and Poesch, G. H., *Ohio Agric. Exp. Sta. Bull.*, 515 (1932).
[2] Post, K., *Cornell Univ. Agric. Exp. Sta. Bull.*, 787 (1942).
[3] Rünger, W., *Licht und Temperatur im Zierpflanzenbau* (Paul Parey, 1957).
[4] Blake, J., *Rep. Fourteenth Int. Hort. Congr. Netherlands*, 331 (1955).

Measurement of Leaf Colour

Leaf colour is a valuable index of nutrient status in flowering plants. Yellowing of the leaves is associated with deficiencies of nitrogen, magnesium, iron, manganese, zinc and molybdenum in many species.

The severity of leaf chlorosis has been recorded by subjective description, by colour photography and by estimation of the chlorophyll content. Chlorophyll estimations yield unambiguous results and have proved most satisfactory in experiments requiring a high degree of accuracy.

In many investigations it is desirable to measure the changes in colour which occur during maturation of the leaf. Chlorophyll estimations involve the destruction of the leaf, and, in consequence, large-scale experiments must be carried out in order to provide an adequate replication in each sample of leaves. As an alternative to this rather laborious procedure, a system of colour matching suitable for attached leaves has been adopted in recent experiments on lime-induced chlorosis, carried out at Sheffield.

A comparator has been made. The colours were prepared by mixing oil paints, which were applied to squares of absorbent paper (thick filter paper is suitable). The colours were arranged in series and matched against the Ridgway[1] colour standards (Table 1). Through the colour series there is a regular change in spectral composition. In order to match chlorotic leaves more easily (they are often bleached in addition to being yellow in colour) the colours at the yellow end of the comparator are of lower intensity.

Colour matching is carried out under standard conditions of white lighting provided by a Siemens-Ediswan Daylight Blue 60-W. lamp. Where leaves show an intervenal chlorosis, the colour matched is that of the intervenal areas.

The error involved in matching leaf colours varies according to the characteristics of the leaf. Species with waxy or glossy leaves are the most difficult subjects. In *Lathyrus montanus* and *Betonica officinalis*, the leaves of which have a matt surface, the error was found not to exceed 5 per cent in the 10–25 range of the colour series and 3 per cent in the 26–33 range.

Even in the standardized environment of a growth cabinet, the rates of leaf appearance in a population of seedlings germinated at the same time are not

synchronous. For this reason, colour-values determined at fixed intervals of time after germination are not comparable. Trials have shown, however, that development of colour in successive leaves can be measured satisfactorily if the timing of the determinations is based on a biological unit of time. The unit is that occurring between the appearance of two successive leaves at the same growing point, that is, in each seedling, the colour of each foliage leaf is measured when its successor first appears and afterwards, at intervals determined by the appearance of successive leaves, at the same growing point. Provided that sufficient records are obtained and the behaviour of the seedlings is fairly uniform with respect to leaf colour, a series of curves or histograms may be constructed to represent the development of leaf colour during the seedling phase (Fig. 1). Where the primary shoot is non-persistent, being replaced by one or more secondary shoots soon after germination, the source of comparable data is limited. The system of colour-matching is unsuitable for grasses because growth is not monopodial and the leaf is not a unit of limited growth as it is in a dicotyledon.

In comparative work on lime-induced chlorosis it has been found possible to construct, for each species,

Table 1. Colour Series used in the Leaf Colour Comparator. Inset is the Range sufficient for most Species

Colour No.	Ridgway colour No.	Name of colour
1 2 3	23*B*	Pale lemon yellow
4 5 6	25*B*	Light greenish yellow
7 8	25*I*	Oil yellow
9	27*I*	Javel green
10		
11 12	29*I*	Cosse green
13 14 15	29*K*	Lettuce green
16 17 18	31*I*	Yellow green
19 20 21	31*K*	Parrot green
22 23	31*M*	Cedar green
24 25 26 27 28	33*I*	Scheele's green
29 30 31 32	34*K*	Grass green
33		
34 35	35*K*	Meadow green
36 37 38	35*M*	Antique green
39	37*M*	Diamine green
40 41	39*M*	Anthracine green
42	41*M*	Myrtle green

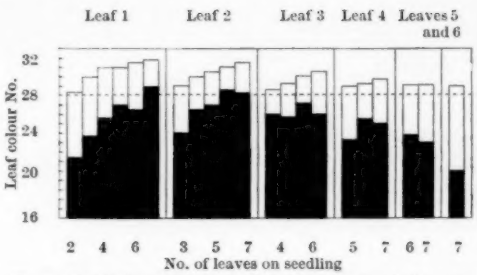

Fig. 1. Leaf colour measurements to show the onset of a permanent lime-induced chlorosis in seedlings of *Betonica officinalis*. Critical boundary line at colour number 28. □, On nutrient-sufficient brown earth; ■, on calcareous soil

a critical boundary-line above which lie the colour development curves from control seedlings on a non-calcareous soil having sufficient nutrients. Values which fall below the boundary indicate some degree of chlorosis. The position of the boundary line varies according to the species, being at 25–26 in *Rumex acetosa*, 26–27 in *Betonica officinalis* and 30–31 in *Trifolium medium*.

Interspecific comparisons may be made in seedlings by examining the timing and magnitude of disparencies between the colour data for leaves of the experimental and control plants. A convenient method of summarizing this type of information is illustrated in Fig. 2.

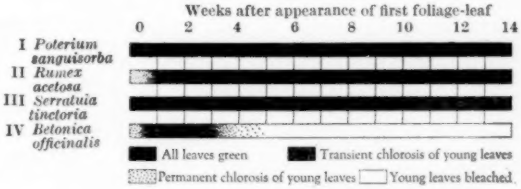
Weeks after appearance of first foliage-leaf
0 2 4 6 8 10 12 14
I Poterium sanguisorba
II Rumex acetosa
III Serratula tinctoria
IV Betonica officinalis
All leaves green
Transient chlorosis of young leaves
Permanent chlorosis of young leaves
Young leaves bleached

Fig. 2. The pattern of colour development in seedlings of four species growing on a calcareous soil. I, No chlorosis; II, transitory chlorosis; III, persistent, non-progressive chlorosis; IV, progressive chlorosis

The method is sensitive to the blue-leaf effects which are associated with an abundant supply of nitrogen. The comparator may not be used to follow the changes in colour in senescent leaves, as these are essentially changes in intensity and may be paralleled by the accumulation of masking piments.

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[1] Ridgway, R., *Colour Standards and Nomenclature* (Washington, D.C., 1912).

Non-Lethal Pathological Infection of Roots

Rovira and Bowen[1] reported reduction in root elongation and in root-hair development following the addition of pasture soil suspension to seedlings of clover, tomato, *Phalaris tuberosa* and *Pinus radiata* raised in sterile agar or sand culture. They suggested the possibility of a complex interaction between plants and micro-organisms. Since there was no observable effect on the plant tops in their experiments, the reduced absorbing surface of the roots was apparently

Fig. 1. Uninoculated control seedlings of *P. radiata* on left; plants on right are taken from liquid culture jar inoculated with *Phytophthora* sp. three months before

offset by the 'luxury' amounts of plant nutrients used.

While continuing investigations on a disease of *P. radiata* caused by *Phytophthora* spp. in New Zealand[2,3], some inoculation results were obtained in which many infected roots were drastically retarded without being killed. While the phenomenon recorded by Rovira and Bowen may have been similar to the stunting of roots and shoots without actual infection as reported by Kerr[4], it may have been due to the condition described here.

Newly germinated seedlings of *P. radiata* were grown in aerated culture solution for three months in a glasshouse. They were then inoculated by adding zoospores of a *Phytophthora* sp. which is widespread in pumice soil supporting a large 30–35-year-old plantation of *P. radiata*. Three weeks after inoculation, roots were found to be infected, being pale brown in colour and covered with mycelium and large numbers of sporangia. After a further three weeks many of the infected primary and secondary roots were dead, particularly in the distal portions of the root systems, but many others had resumed apical growth or were producing new lateral roots through infected cortical tissues. New root growth was very restricted by comparison with uninoculated check plants. After a further six weeks new root growth was still sparse and top growth of living plants was markedly depressed (Fig. 1). Shoot length was reduced by a third and the length of scale leaf by a fifth of that in the controls. The non-lethal infection and stunting of roots was obtained in aerated and non-aerated nutrient culture solutions or in tap water replacing the original culture solution. The pathogen was re-isolated from portions of retarded root.

Microtome sections of retarded roots showed that mycelium was confined to the cortex, being intracellular and reaching, but not penetrating, the endodermis. In dead roots mycelium had invaded all tissues, with occasional oospores occurring in the vascular cylinder.

Similar results were obtained in a further test with the same and other *Phytophthora* spp., including *P. cinnamomi* and *P. cactorum*. In some experiments infection resulted in death of complete root systems.

A significant feature of the results is that in the absence of control plants many of the diseased root systems would pass as healthy, the pale brown roots with infected cortical tissues being outwardly similar to roots undergoing normal suberization. In the field, where there are no controls to give a measure of root stunting, it is common to find rootlets with dark cortical tissues and white vascular cylinders which are still functional, judging by the possession of white elongating root tips.

Phytophthora spp. have been found to be widespread in New Zealand, in cultivated soil, exotic pine plantations and indigenous forests. If they and other soil fungi, in addition to being active pathogens on some plants, are affecting less-susceptible hosts in a manner similar to that described here, they must be regarded as constituting an important but not easily recognized biotic factor of the environment. In this role they could, for example, be of significance in some of the puzzling decline diseases in which there is an apparent lack of primary symptoms. They could also be predisposing agents in connexion with insect or fungus attack on upper parts of plants where low moisture content is a governing factor. By affecting host vigour they could influence ecological succession in natural plant communities.

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[1] Rovira, A. D., and Bowen, G. D., *Nature*, **185**, 260 (1960).
[2] Newhook, F. J., *N.Z. J. Agric. Res.*, **2**, 808 (1959).
[3] Sutherland, C. F., Newhook, F. J., and Levy, J., *N.Z. J. Agric. Res.*, **2**, 844 (1959).
[4] Kerr, A., *Austral. J. Biol. Sci.*, **9**, 45 (1956).

Wettability and the Capture, Carriage and Deposition of Particles by Raindrops

During rain the particulate matter of the atmosphere is washed to the ground, and it has been suggested that the wettability of the particles affects the efficiency with which they are picked up by the falling droplets. The rain-wash of fungal spores which are not easily wetted has been reported, however, by Gregory[1] and Hirst[2]. It was therefore decided to investigate the effect of surface wettability on the manner in which fungal spores are captured, carried and deposited by water droplets. The investigation was carried out with the simplest of apparatus and by direct observation under the microscope.

Capture and carriage. Water droplets approximately 2 mm. in diameter were caused to fall distances between 10 cm. and 25 cm. so that they then swept the conidiophores of *Verticillium alboatrum* or *Penicillium chrysogenum* before being caught in a mixture of oil and 'Vaseline'.

Droplets which swept *Verticillium* conidiophores invariably contained spores which exhibited Brownian

motion within the droplet. No spores were ever observed within the droplets which had swept *Penicillium* conidiophores; but the spores were always observed on the surface of the droplet and most frequently at the boundary where the droplet rested on the oil and 'Vaseline' mixture. Evidently the readily wettable *Verticillium* spores enter the droplets with ease, whereas with the *Penicillium* spores, with their water-repellent surfaces and under the conditions of these experiments, impact velocities between spores and droplets were too low to overcome the interfacial energy at the surface of the droplets.

Since electrostatic charges have been shown to occur on raindrops by Chalmers[3] and on spores in air by Gregory[4], the possibility of an electrostatic attachment between water droplets and spores with water-repellent surfaces was investigated.

A mass of *Penicillium* spores held on the point of a steel needle was brought into contact with a water droplet produced at the tip of a hypodermic needle and subsequent events observed under the microscope. With both needles connected to earth, that with the spores was slowly moved away from the droplet. As this was done, it was observed that the part of the droplet in contact with the spores became drawn out, until finally, when contact ceased, the droplet assumed its original shape and a number of spores was left adhering to its surface.

Since both droplet and spores were earthed, it must be concluded that despite the water repellency of the spore surface, the spores were held to the droplet surface by molecular adhesion.

The manner in which fungal spores are captured and carried by rain or spray droplets is influenced by the wettability of the spore surface. The readily wettable spores of *Cephalosporium*, *Fusarium*, *Verticillium* and *Pullularia* enter droplets with ease and are carried within. At low impact velocities the 'non-wettable' spores of *Aspergillus*, *Cladosporium* and *Penicillium* are captured by adhering to the surface of the droplet.

Deposition of spores on water-repellent surfaces. Since most vegetation has water-repellent surfaces, it was decided to investigate whether surface wettability affected the deposition of spores by water droplets. In an initial experiment a microscope slide was covered on one surface with a mixture of 'Vaseline' and paraffin wax. A water droplet with its surface densely covered with 'non-wettable' *Penicillium* spores was placed at one end on the prepared surface, and the slide inclined and sharply tapped so that the droplet ran over it. As the droplet moved, most of the spores became attached to the hydrophobic surface and were visible to the unaided eye as a linear 'stain' along the course taken by the droplet. When the procedure was repeated with wettable *Verticillium* spores, and the slide examined under the microscope, no spores were seen in the track over which the droplet moved. When the experiment was repeated using plant leaves and the spores of other fungi, similar results were obtained.

It may be concluded, therefore, that when a droplet carrying spores falls on and moves over a hydrophobic plant surface, any 'non-wettable' spores brought into contact with the surface will tend to be deposited to the rear of the moving droplet, whereas wettable spores will either be washed away or deposited where the droplet comes to rest. During rain, the process is complicated by the splash-dispersal mechanism described by Gregory *et al.*[5] and the fact that through contact with water the wettability of a hydrophobic surface increases (Adam[6]).

Water droplets falling on hydrophobic leaf surfaces do not form a continuous film, but, to quote an expression of Adam, "pearl away in beads". It was observed that when a succession of droplets from a point source fell on a blade of grass, the beads did not run along the axis of the blade but 'pearled' off the edges, frequently alighting on an adjacent blade. This pearling away of water in beads from hydrophobic plant surfaces may in itself act as a spore dispersion mechanism distinct from the splash mechanism of Gregory *et al.*[5].

While it has not been investigated, in the removal of a radioactive dust cloud by rain, a 'non-wettable' fraction might tend to be deposited and retained on the surfaces of vegetation.

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[1] Gregory, P. H., *Trans. Brit. Mycol. Soc.*, **35**, 1 (1952).
[2] Hirst, J. M., *Trans. Brit. Mycol. Soc.*, **36**, 375 (1953).
[3] Chalmers, J. A., *Atmospheric Electricity* (Pergamon Press, 1957).
[4] Gregory, P. H., *Nature*, **180**, 330 (1957).
[5] Gregory, P. H., Guthrie, E. J., and Bunce, M. E., *J. Gen. Microbiol.*, **20**, 328 (1959).
[6] Adam, N. K., *Endeavour*, **17**, 37 (1958).

Taxonomy of *Xanthomonas* and *Pseudomonas*

Application of computers to problems of bacterial taxonomy has been suggested by Sneath[1], who described a method for handling large quantities of taxonomic data by an electronic computer. By this type of analysis, an outline of classification based on equally weighted features is obtained.

Hill[2] carried out an electro-taxonomic analysis of the *Staphylococci* and determined *Staphylococcus aureus*, *S. saprophyticus*, and *S. roseus* to be natural groups, whereas *S. lactis* and *S. afermentans* were not, since individual strains of these 'species' proved to be as different from each other as from the previous species. Other taxonomic studies involving the use of a computer[3] have yielded equally interesting results.

Work on the classification and taxonomy of the *Pseudomonas*, *Achromobacter*, *Flavobacterium* groups using the IBM 650 magnetic drum computer is being carried on in our laboratory[4,5]. The method clearly indicates generic and supra-generic groupings and there are indications, from our results and from others reported[6,7], that the genus-level may lie approximately at 60 per cent and the species-level at 75 per cent *S* ('similarity value'), meaning that organisms of the same genus will share about 60 per cent of their characters and strains will share 75 per cent or more of their characters.

Since *Xanthomonas* is distinguished from *Pseudomonas* by production of a yellow insoluble pigment and by plant pathogenicity, and since some *Pseudomonas* are plant pathogens, we became interested in applying the Adansonian method in an analysis of representatives of these groups to determine whether

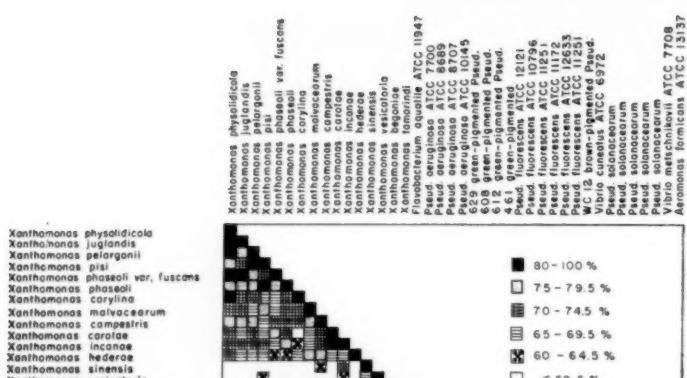

Fig. 1. Full table of S-values obtained from the analysis of forty representative strains, predominantly *Xanthomonas* and *Pseudomonas*

the separation of *Xanthomonas* from *Pseudomonas* is real or artificial (that is, taxonomic or determinative).

Some well-defined species of *Xanthomonas*, strains of *Pseudomonas solanacearum* which had been isolated from widely different geographical sources, and a number of named and freshly isolated *Pseudomonas* species were studied. All strains were tested, using standard bacteriological methods[8], in our laboratory and the 134 characteristics or 'states' obtained for each culture were punched on data cards. Calculations of S-values were performed by machine. The full S-table results are shown in Fig. 1.

It appears that three groupings were obtained: *Xanthomonas physalidicola* to *Flavobacterium aquatile*: *Pseudomonas aeruginosa* ATCC 7700 to *Vibrio cuneatus*; and the *Pseudomonas solanacearum* strains. *Vibrio metschnikovii* and *Aeromonas formicans* fall completely outside the *Pseudomonas–Xanthomonas* groups, in accord with results of other analyses[8].

The *Xanthomonas* species fall very clearly into two related groups which we have called *Xanthomonas* I and *Xanthomonas* II, for convenience. *Xanthomonas* II, the small group, contains only four species: *X. sinensis*; *X. vesicatoria*; *X. begoniae*; and *X. tamarindi*. All other species of *Xanthomonas* fall into Type I. However, within Type I, there is a small, tight clustering of the species *X. physalidicola* to *X. phaseoli*, which are so clearly related that they seem to form a 'taxonomic' species (intra-group $S = 78$ per cent). The five *Pseudomonas solanacearum* strains form a closely related group, quite distinct from *Xanthomonas* types, and probably constitute a true taxonomic species (intra-$S = 75$ per cent).

The fluorescent *Pseudomonas* form a large homogeneous group (intra-$S = 69$ per cent) within which three sub-groups can be delineated: a *Pseudomonas fluorescens* group and two *Pseudomonas aeruginosa*-like groups, one psychrophilic (461 to 629), consisting of organisms from marine sources and the other mesophilic, including all the ATCC *P. aeruginosa* cultures.

The two *P. aeruginosa* groups are so closely related (inter-$S = 74$ per cent) and individually so homogeneous (intra-$S = 75$ per cent and 85 per cent, respectively) that they could represent a single species with two varieties.

The maximum values for the inter-group S-values are shown in Fig. 2, the groups having been arranged according to the computed inter- and intra-S-values. It can be noted that *Xanthomonas* links with the *aeruginosa–fluorescens* block by a group relationship between the latter and *Xanthomonas* I, at a level close to the possible genus-level. *Xanthomonas* II is closely related to *Xanthomonas* I, but not to the principal *Pseudomonas* group. Indeed, it is interesting to note that *Xanthomonas* II has a nearly generic relationship with the ATCC *Flavobacterium aquatile*, which may not, however, be a *Flavobacterium*, since it has polar flagella.

As shown in Fig. 2, the *Pseudomonas solanacearum* species, which themselves produce a brown pigment, are linked to the *Pseudomonas* group through relationship with a sea-water-requiring brown-pigmented *Pseudomonas* species, but differ as much, or more, from the other *Pseudomonas* tested, as did the *Xanthomonas* species.

A more complete presentation of these results, with a discussion of the characteristics separating the groups, will be published in full elsewhere.

The *Xanthomonas* species were supplied by Dr. M. P. Starr, Department of Bacteriology, University of California, Davis, and the *Pseudomonas solanacearum* isolates by Dr. A. Kelman, Plant Pathology Department, North Carolina State College, Raleigh. The named strains of *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Vibrio metschnikovii*, *Aeromonas formicans* and *Flavobacterium aquatile* were obtained from the American Type Culture Collection, Washington, D.C. Other pseudomonads were freshly isolated in our laboratory from marine sources.

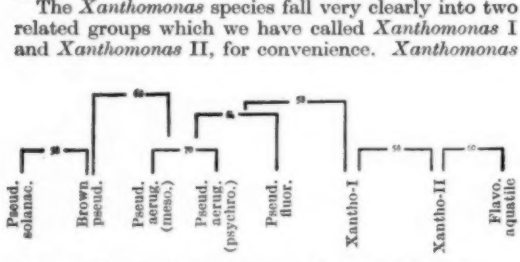

Fig. 2. Diagrammatic representation of inter- and intra-group S-values indicating group relationships

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[1] Sneath, P. H. A., *J. Gen. Microbiol.*, **17**, 184, 201 (1957).
[2] Hill, L. R., *J. Gen. Microbiol.*, **20**, 277 (1959).
[3] Sneath, P. H. A., and Cowan, S. T., *J. Gen. Microbiol.*, **19**, 551 (1958).
[4] Colwell, R. R., and Liston, J., *App. Microbiol.*, **8**, 104 (1960).
[5] Liston, J., and Colwell, R. R., *Bacteriol. Proc.*, 78 (1960).
[6] Colwell, R. R., and Liston, J. (work to be published).
[7] Cheeseman, G. C., and Berridge, N. J., *J. App. Bacteriol.*, **22**, 307 (1959).
[8] Soc. Amer. Bacteriol., *Manual of Microbiological Methods* (McGraw-Hill Book Co., Inc., New York, 1957).

Effects of Kinetin and Indole-3-acetic Acid on Multiplication Rates of *Paramecium*

Kinetin (6-furfuryl aminopurine) is known to cause division in certain plant cells which could not otherwise divide[1]. But other cases are reported in which the rate of cell division is decreased after its application, for example, those of De Ropp[2], Kemp *et al.*[3], Pilet *et al.*[4], and Ham *et al.*[5]. How this substance affects the rate of division of animal cells is a question of interest. In a recent report[6], Guttman and Back re-analysed data and repeated experiments in which they had previously[7] found that kinetin causes an increase in the rate of division of *Paramecium caudatum* when added in low doses to the culture medium. Their later work[6] indicated that stimulation of cell division was present, but on a lower level than that previously reported. Coincident with the appearance of the second report of Guttman and Back, we reported elsewhere[8] the results of experiments in which kinetin, when used at the same concentrations and under similar conditions, was found to be inhibitory to division of *P. caudatum*.

Table 1 shows the results of a series of experiments in which comparisons were made between controls and treated *Paramecia* which were immediate descendants of one individual of a clone. In every series, there was inhibition of division at a significant level of confidence in kinetin-treated *Paramecia* as compared with untreated controls.

A smaller series of comparisons was made between rates of division in *Paramecia* treated with kinetin plus indole-3-acetic acid (IAA), and untreated controls. The results are given in Table 1. The results show that IAA appears to reverse the inhibition of division produced by 0·5 p.p.m. kinetin, and to counteract the severity of the inhibition produced by 1·0 and 1·5 p.p.m. kinetin. Antagonism between kinetin and IAA has been found in other experiments, for example, those of Wickson and Thimann[9] in lateral bud development.

Table 1. Mean Numbers of Divisions per 24-hr. Period of *Paramecia* treated with Kinetin (K) alone or in Combination with Indole-3-acetic Acid, as compared with Untreated Controls

No. of 24-hr. comparisons	Control	*S.E.*	Treatment solution (p.p.m.)			*t* value	Level of significance
			0·5 K.	1·0 K.	1·5 K.		
64	0·843	0·47	0·742			1·7	0·1
60	0·850	0·51		0·650		2·99	0·01
61	0·819	0·49			0·606	3·31	0·01
			0·5 K. +2 IAA	1·0 K. +2 IAA	1·5 K. +2 IAA		
41	0·607	0·48	0·607				
42	0·619	0·48		0·548		0·939	0·35
41	0·610	0·23			0·512	1·271	0·25

Guttman and Back[6] re-analysed their results, recording division frequency for each day separately, their results showed a day-by-day pattern of divisions in kinetin-treated *Paramecia* which was approximately the same as the pattern for the controls, but at a higher level. Re-analysis of our Table 1 by this method, recording division frequency for each day separately, showed that the up-and-down pattern of the mean numbers of divisions for kinetin-treated *Paramecia* is the reverse of that for the control *Paramecia* in every case excepting for the third day in *Paramecia* treated with 0·5 and 1·0 p.p.m. kinetin. These show a decrease from day 2 to day 3, similar to the decreasing pattern for the controls. The re-analysis of the data on *Paramecia* treated with kinetin + IAA is shown in Fig. 1. The division pattern of the treated *Paramecia* is the reverse of the pattern for controls. Although the addition of IAA to the kinetin medium apparently reversed the inhibitory action of kinetin alone when the overall means for the three days are considered, day-by-day analysis shows that the reversal did not occur until day 2.

Woodruff[11], in numerous studies of division in *Paramecia*, found a regular cyclic rhythm of increase and decrease under normal conditions. The controls in our experiments and in those of Guttman and Back show day-to-day differences which might be part of such a cyclic rhythm, or which might be due to change in rate on transfer to a new medium.

In our experiments, the up-and-down rhythm pattern of division of *Paramecia* treated with kinetin was the reverse of the pattern for controls, that is, the number of divisions increased in treated *Paramecia* when it decreased in the controls, and vice versa. This also does not agree with the results of Guttman and Back, who found that the pattern of division for kinetin-treated *Paramecia* was similar to the pattern for the controls, with parallel increase and decrease in the number of divisions in all cases.

Guttman[12] in 1956 reported a stimulatory effect of kinetin on mitosis in cells of intact onion roots. Her data were obtained by counting mitotic figures in random fields of squash preparations. In similar work in which mitoses were counted in serial longitudinal sections of onion root tips, McManus[13] found an

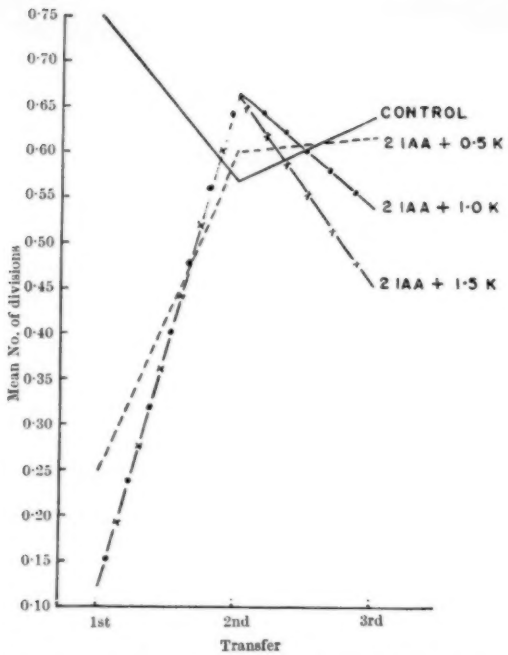

Fig. 1. Cell division in *Paramecium caudatum* treated with indole-3-acetic acid plus kinetin (parts per million). The influence of treatment on division frequencies after each of three daily transfers to new medium

inhibitory effect of kinetin, at comparable concentrations. Inhibition was also indicated by data obtained by Jensen and Pollock[14] in quantitative work on the deoxyribonucleic acid content of onion root tips treated with kinetin.

Whether or not kinetin occurs naturally in plant and animal tissue, clarification of the true nature of its effect on cell division under various conditions is of importance to our understanding of the growth process. Undoubtedly, interactions between kinetin and IAA and between them and other substances and events within the cell, which compel its division, must be very complex.

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[1] Miller, C. O., Skoog, F., Okumura, F. S., Von Saltza, M. H., and Strong, F. M., *J. Amer. Chem. Soc.*, **77**, 2662 (1955).
[2] De Ropp, R. S., *Plant Physiol.*, **31**, 253 (1956).
[3] Kemp, H. T., Fuller, R. G., and Davidson, R. S., *Science*, **126**, 1182 (1957).
[4] Pilet, F., Hildebrandt, A. C., Riker, A. J., and Skoog, F., *Amer. J. Bot.*, **47**, 186 (1960).
[5] Ham, R. G., Eakin, R. E., Skinner, C. G., and Shive, W., *J. Amer. Chem. Soc.*, **78**, 2648 (1956).
[6] Guttman, R., and Back, A., *Science*, **131**, 986 (1960).
[7] Guttman, R., and Back, A., *Nature*, **181**, 852 (1958).
[8] McManus, Sr. M. A., and Sullivan, K., *Proc. Iowa Acad. Sci.*, **67**, 501 (1960).
[9] Wickson, M., and Thimann, K. V., *Plant Physiol.* (Supp.), **31**, 28 (1956).
[10] Lansford, jun., E. M., Skinner, C. G., and Shive, W., *Arch. Biochem. Biophys.*, **73**, 191 (1958).
[11] Woodruff, L. L., and Baitsell, G., *J. Exp. Zool.*, **11**, 339 (1911).
[12] Guttman, R., *Chromosoma*, **8**, 341 (1956).
[13] McManus, Sr. M. A., *Nature*, **185**, 44 (1960).
[14] Jensen, W. A., and Pollock, E. G., *Plant Physiol.* (Supp.), **33**, 15 (1958).

Cycle and Rhythm in the Ovulation of the Spider *Latrodectus geometricus* C. Koch

We define 'cycle' as that phenomenon represented by regular variations of internal origin. By 'rhythm', we understand a phenomenon represented by regular variations imposed by external conditions. 'Regularity' signifies a constancy of the interval separating two identical stages of the variation[1]. The ovulation of the cosmotropical spider *Latrodectus geometricus* C. Koch is at one time both cyclic and rhythmic.

Under laboratory conditions, with regular nutrition and prior fecundation, the adult *Latrodectus* lays an average of 22·56 cocoons at an interval of one every four days during the first part of its life. This interval becomes progressively longer until death intervenes. This metabolic ovarian cycle, established by observation of the laying of some thousands of cocoons, is illustrated in Fig. 1, which represents the percentage frequency (ordinate) of different time-intervals in days separating the laying of successive cocoons (abscissa)[2].

86·8 per cent of the cocoons are laid between 0230 and 0320 hr., with the relative maximum falling at 5.30. Under natural conditions this maximum is more sharply accentuated. Fig. 2 represents the observations made on several hundred cases, in which the numbers of cocoons produced (ordinate) are grouped against intervals of 1 hr. (abscissa).

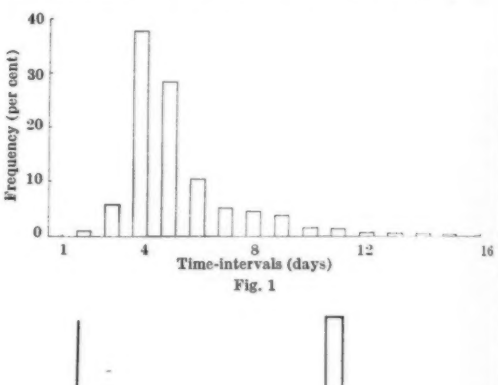

Fig. 1

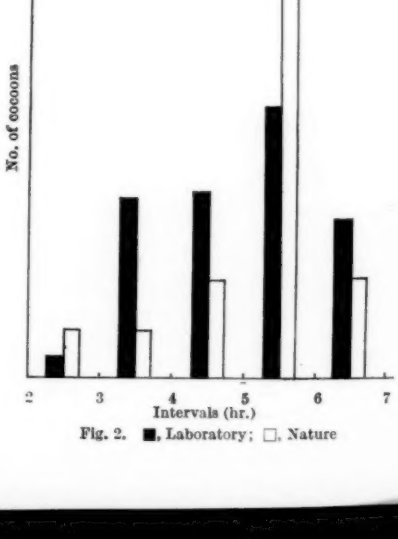

Fig. 2. ■, Laboratory; □, Nature

Observations on the production of 2,430 cocoons under varying controlled conditions (alternation of day and night with a displacement of 12-hr., period of daily illumination, temperature, humidity, time of feeding) have shown the only factor capable of influencing this rhythm, superimposed on the ovulation cycle, to be nycthemeral. By alternating the day and night cycle, the maximum intensity of ovulation can be shifted to fall between 1700 and 1800 hr., that is to say before an instantaneous change from obscurity to light, but the laying period is, however, more spread out than under normal conditions, 66 per cent of the cocoons being laid between 1400 and 2200 hr.[3].

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[1] Reinberg, A., and Ghata, J., *Rythmes et Cycles biologiques* (Paris, 1957).
[2] Bouillon, A., *Studia Univ. Lovanium, Fac. Sci.*, **1**, 10 (Leopoldville, 1957).
[3] Bouillon, A., and Lekie, R., *Studia Univ. Lovanium, Fac. Sci.*, **12**, 5 (Leopoldville, 1960).

Significance of the Regeneration of Lateral Plates of the Stickleback (*Gasterosteus aculeatus* L.)

The taxonomy of the stickleback (*Gasterosteus aculeatus* L.) has excited the interest of zoologists for more than two centuries. Even now there are many unexplained questions concerning the differentiation within the species. The most difficult to explain is the variable number of lateral plates covering the body of this fish.

My earlier investigation, already described[1], of the process of regeneration of the lateral plates seems to question the popular conception that small losses in plates are caused by the mechanical tearing off of the plates in natural conditions. When the lateral plates of the stickleback were removed with the cutis, then the connective tissue, the subcutaneous tissue and a thin layer of muscles were regenerated by the organism by differentiation of the mesenchyme tissue. Plates removed from a considerable area of the body regenerated at the same time. This excludes the possibility of their being regenerated only in the proximity of the untouched plates. The discovery of the regeneration of the lateral plates which had been cut away completely is interesting, because so far all experiments carried out with dermal bones (which have the same structure and origin as lateral plates) have shown that regeneration takes place only when they were cut away only partially[2–5].

Continuing the investigation has shown recently that the regeneration of the amputated parts of the body of the stickleback sets in relatively quickly. The epidermis fills up the losses of the parts of the body removed within six days. In about fifteen days connective tissue penetrates in between the epidermis and the regenerating muscles. After 30 days the connective tissue differentiates into loose, elastic and subcutaneous kinds of tissue. A germ of a lateral plate appears on about the fiftieth day of the regenerating processes. Between the 100th and 150th day of regeneration, the lateral plate is usually restored, but sometimes its shape is irregular. In the case of a partial amputation of lateral plates, restoration of the loss takes place more quickly; this is natural, because the regenerated part profits by the cells of the old tissue.

It seems that this investigation throws some light on the complicated problem of the individual mutability of stickleback which is conditioned, among other factors, by a variable number of lateral plates.

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[1] Penczak, T., *Zeszyty Naukowe* U.L. (Łódź), Ser. II, *Nauki Mat. Przyrod.*, **7**, 123 (1960).
[2] Beigel, C., *Bull. Int. Acad. Pol.*, Cl. Mat. Nat. (Cracovia), B, 655 (1910).
[3] Sauter, M., *Arch. Entw. Mech.*, **132**, H. 1 (1934).
[4] Suworow, E., *Podstawy ichtiologii*, 304 (PWN, Warszawa, 1954).
[5] Wunder, W., *Umschau in Wissenschaft und Technik*, **3** (Leipzig, 1953).

Spiracle Control in Dragonflies

Hoyle[1] has shown that carbon dioxide has a direct action on the neuro-muscular junction of the closer muscle of the second spiracle of the locust, reducing the electrical responses and the tension developed in spite of maintained stimulation of the motor axons in the transverse nerve. However, this peripheral control mechanism does not seem to play an important part in the regulation of the intact spiracle which, except during flight, remains firmly synchronized with ventilation[2]. It might be expected to play a more important part in an insect the spiracles of which are not always synchronized with ventilation, such as the dragonfly. The adults of four species of Aeschnid dragonflies, occurring in Great Britain, have been examined and none shows ventilation synchronization in spiracles 1, 2 or 3. In the 15 species of Libellulidae from Great Britain and Uganda which have been examined, however, spiracle 1 opens with abdominal inspiration and closes completely or partially with expiration: at rest, spiracles 2 and 3 remain closed, but they may join spiracle 1 as inspiratory spiracles for a brief period after struggling or flight.

Spiracles 1, 2 and 3 are similar to the abdominal spiracles in possessing a closer muscle and in being opened by a cuticular elastic mechanism. The activity of the closer is controlled by the interaction of two mechanisms. In the first a central reflex, initiated by any movement—usually flight—inhibits the streams of motor impulses in the median nerves to spiracles 1, 2 and 3 so that they open instantaneously. They remain open throughout flight. The second mechanism depends on the peripheral action of carbon dioxide, and the site of action is most probably the neuro-muscular junction of the closer as in the locust[1]. Oscilloscope records show that the pattern of motor impulses in the transverse nerve to the closer muscle remains unaltered while the spiracle opens to locally administered 5 per cent carbon dioxide. In Aeschnids the spiracle opens with irregular fluttering movements, but in Libellulids it starts immediately to make movements synchronized with ventilation, although the other member of the pair remains closed. Each spiracle of the pair receives identical motor input, since the axons in the median nerve divide into right and left transverse nerves. In Libellulidae the motor input nearly always shows a rhythm, increasing in frequency with expiration and decreasing with inspiration. The rhythm is very marked when ventilation is strong,

but may disappear during weak ventilation. In the absence of carbon dioxide, the lower (inspiratory) frequency is adequate to maintain a tetanus in the closer and the spiracle stays shut. Local administration of 5 per cent carbon dioxide to the spiracle makes the lower frequency ineffective so that the spiracle opens during inspiration and recloses with expiration. In 5–10 per cent carbon dioxide the higher frequency also becomes partially or completely ineffective so that the spiracle remains open. Carbon dioxide in the thoracic ganglia stimulates abdominal ventilation and this in turn gives a more strongly marked rhythm in the thoracic transverse nerves. It has been shown that, unlike the locust, there is no rhythmic ventilation centre in the thorax and, after removal of the abdomen, treatment of the thoracic ganglia with carbon dioxide does not alter the patterns of motor impulses in the transverse nerves. The reaction can take place only via the abdomen.

In this way the insect possesses a facultative synchronization mechanism which allows the spiracles to be coupled to the ventilatory rhythm in the presence of low concentrations of carbon dioxide, and it is clear that the peripheral control mechanism plays an important part. Reduced oxygen tensions have no comparable affect. Possibly similar facultative spiracle synchronization has been observed in the cockroach[3], and such mechanisms in which variation takes place peripherally, while the contribution from the central nervous system remains largely unaltered, may reflect the need for the arthropod nervous system to economize in central connexions[4].

The sensitivity of the response of the spiracle muscle to carbon dioxide has been shown to depend on the state of hydration of the insect. In partially desiccated dragonflies the spiracles do not open in less than about 5 per cent carbon dioxide in air, and after flight they close immediately. In well-hydrated insects they open in 1–2 per cent carbon dioxide, and they may remain open for as long as 2 min. after flight. Experimentally produced variations in the osmotic pressure of the hæmolymph have been shown to alter the spiracle muscle's sensitivity directly, both before and after denervation, so that internal osmometers relaying to the central nervous system need not be postulated, and again the reaction takes place entirely peripherally.

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[1] Hoyle, G., *J. Insect Physiol.*, **4**, 63 (1960).
[2] Miller, P. L., *J. Exp. Biol.*, **37**, 264 (1960).
[3] Hazelhoff, E. H., *Z. vergl. Physiol.*, **5**, 179 (1927).
[4] Wiersma, C. A. G., *Cold Spring Harbor Symposia Quant. Biol.*, **17**, 155 (1952).

MICROBIOLOGY

Occurrence of *Salmonellae* in Domestic Animals and Poultry in India

This communication presents the results of an investigation into the occurrence of *Salmonellae*, usually in the symptomless carrier-state, in domestic animals and poultry in the State of Uttar Pradesh in India.

Bacteriological examination was mainly confined to fæcal samples; however, small pieces of liver, spleen, gall-bladder, lungs and intestine with its contents were also collected from domestic animals and poultry for examination.

The methods of isolation of *Salmonellae* used were on the lines adopted by Smith and Buxton[1] and Edwards *et al.*[2] with minor modifications. *Salmonella* genus-specific 0-1 bacteriophage was also used as advocated by Cherry *et al.*[3]. The serological typing was done by one of us at the Armed Forces Medical College, Poona. The strains were also sent to Dr. P. R. Edwards, Chamblee Georgia, U.S.A., and to Dr. Joan Taylor, Salmonella Reference Laboratory, London, for serological typing.

A total number of 2,970 domestic animals including poultry (1,332 fowls; 206 dead in shell embryos; 68 pigeons; 110 ducks; 455 cattle; 290 buffaloes; 163 sheep; 91 goats; 247 pigs and 18 rats) were examined during the course of the investigation. The occurrence of *Salmonella* species was demonstrated in 24 fowls, 7 dead in shell embryos, 1 duck, 8 cattle, 9 buffaloes, 4 goats, 3 pigs and 1 rat, making a total of 57 isolations.

Seventeen species of *Salmonellae*, including a new sero-type encountered during this investigation, are as follows (the figures in brackets indicate the number of isolations):

Fowls: *anatum* (2); *champaign* (2); *chester* (1); *krittingfoss* (2), *matopeni* (1); *pomona* (1); *richmond* (3); *san-diego* (1); *stanley* (1); *weltevreden* (10).
Dead in shell-embryos: *richmond* (7).
Duck: *san-diego* (1).
Cattle: *aba* (1); *charity* (1); *magwa* (2); *matopeni* (1); *pomona* (2); *mathura* (1).
Buffaloes: *anatum* (1); *enteritidis* var. *jena* (1); *magwa* (1); *pomona* (1); *typhimurium* (1); *weltevreden* (4).
Goats: *magwa* (1); *matopeni* (1); *weltevreden* (2).
Pigs: *anatum* (1); *newport* (1); *Stanley* (1).
Rats: *anatum* (1).

A new species of *Salmonella*, which has been named *Salmonella mathura* with antigenic formula (9) 46 : *i* : *e*, *n*, *z* 15, has been isolated from cattle. The biochemical behaviour of this species, as studied by Dr. F. Kauffmann, International Salmonella and Escherichia Centre, Copenhagen, is as follows: adonitol, rhamnose, salicin, inositol, lactose and sucrose: negative after 30 days.

Positive reaction after 1 day in: dulcitol, sorbitol, arabinose, xylose, maltose, mannitol, glucose and trehalose. Gas production in mannitol and glucose. Stern's glycerolfuchsin broth negative after 8 days. Hydrogen sulphide in ferrous chloride gelatin, negative. Gelatin, negative.

Ammonium glucose and ammonium citrate, positive. Potassium nitrate, positive; Voges-Proskauer, negative; methyl-red, positive; urea, negative.

d-tartrate, +1; *l*-tartrate, delayed and irregular; *i*-tartrate, +2; sodium citrate, +2; mucate, +1.

It is interesting to note that several of the species which probably have been isolated so far only from human beings are being encountered in animals. The importance of such occurrence from the epidemiological and public health point of view is obvious and further work is in progress. Investigation is also being conducted for the further characterization of the new species, *S. mathura*.

We are indebted to Major L. D. Sachdeva, of the Armed Forces Medical College, Poona, for the help and guidance in sero-typing the cultures, and Dr. Joan Taylor, of the Salmonella Reference Laboratory, Colindale, London, and Dr. P. R. Edwards, Chamblee Georgia, U.S.A., for sero-typing of the strains. We gratefully acknowledge the kind help of Dr. William B. Cherry, Chamblee Georgia, U.S.A., for

supplying 0–1 phage, and of Dr. J. B. Shrivastava, National Salmonella Centre, Kasauli, Punjab, for his help, and Dr. F. Kauffmann, International Salmonella and Escherichia Centre, Copenhagen, for final confirmation of the new species, and to Sri C. V. G. Choudary, principal of the Veterinary College, Mathura, for providing the necessary facilities.

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1 Smith, H. W., and Buxton, A., *Brit. Med. J.*, i, 1478 (1951).
2 Edwards, P. R., Bruner, D. W., and Moran, A. B., *J. Infect. Dis.*, **83**, 220 (1948).
3 Cherry, W. B., Davis, B. R., Edwards, P. R., and Hogan, R. B., *J. Lab. Clin. Med.*, **44**, 51 (1954).

Transfer of Virulence in *Rhizobium trifolii*

So far, very few reports concerning transformations in *Rhizobium* have appeared. Krasilnikov[1] reported as early as 1941 that non-infective strains turned infective when they were grown for several months in a filtrate of a highly infective strain. By ultrasonic treatment and with preparations of deoxyribonucleic acid (DNA) Balassa[2,3] has obtained transformations concerning several properties, for example, infective ability under formation of new host specificity, increase or decrease in nitrogen-fixing ability, variation in colony types, and changes in antigenic structure.

I happened to meet with a case of transformation in *Rhizobium trifolii*. As part of a study of the infection process a polysaccharide was prepared from an infective strain[4]. As it had been observed (unpublished results) that inoculated plants formed nodules 1-2 days earlier when this preparation was added, the polysaccharide was added to plants inoculated with a non-infective strain to see whether it might help the avirulent bacteria to overcome the infection barrier.

The clover strain *A* used in the experiment described here has been studied thoroughly by Nutman[5,6]. The original strain lost its infection ability in 1940 (called Bart *A*), but a virulent line (*A* 1) was contained in an isolate from a nodule produced by the original strain. *A* 11 is an avirulent and *A* 121111 a virulent line, isolated from *A* 1. 226, the other clover strain, is a Swedish strain isolated by Bjälfve and so far as known not related to the *A* strain.

A polysaccharide from strain 226 was prepared[7] from a 2-wk.-old culture (Dorn's A_5-medium[8]) by precipitating the whole culture in alcohol, dissolving the precipitate in water and separating the cells by centrifugation. The clear solution was deproteinized according to Sevag[9], precipitated again, and dialysed against distilled water. After another precipitation, the preparation was dried *in vacuo* and a white water-soluble material was obtained.

Seeds of *Trifolium repens* (var. Morsö, Svalöf, Sweden) were germinated aseptically in tubes containing slopes of Jensen's seedling agar. After 2 days, they were inoculated with a few drops of a suspension of *Rhizobium* (Table 1, series 1–8). Immediately after inoculation, 2 mgm. of the 226 polysaccharide in water solution was added. The

Table 1. Plants of *Trifolium repens* inoculated with Avirulent (Bart *A* and *A* 11) and Virulent (*A* 121111 and 226) Clover Bacteria without (−) and with (+) Polysaccharide Preparation from Strain 226 (2 mgm./tube). Each tube contained 10 ml. of Jensen's Agar. The Number of Nodules are Mean Values of Five Replicants

Series No.	Bacterial strain	Poly-saccharide addition	Appearance of the first nodule in days after inoculation	Mean number of nodules per tube after 2 wk.	3 wk.	4 wk.
1	Bart *A*	−	−	−	−	−
2	*A* 11	−	−	−	−	−
3	*A* 121111	−	10	3·3	8·5	12·5
4	226	−	9	2·7	7·0	10·0
5	Bart *A*	+	9	2·5	7·3	9·3
6	*A* 11	+	9	3·5	9·0	12·0
7	*A* 121111	+	7	6·7	12·0	15·0
8	226	+	7	5·8	8·5	10·3
9	Bart *A*	−	−	−		
10	Bart *A*·2	−	8	4·2		
11	Bart *A*	+	8	6·4		
12	Bart *A*·2	+	6	7·4		

appearance of the first nodule in days after inoculation and the number of nodules after 2, 3 and 4 wk. are recorded in Table 1.

Bacteria from the nodules produced by the formerly avirulent Bart *A* in the presence of the polysaccharide were isolated (Bart *A*·2). The colonies had the smooth appearance of 226, not rough like Bart *A*.

New seedlings were inoculated with Bart *A* and Bart *A*·2 with and without polysaccharide 226 (Table 1, series 9–12). From this experiment it is evident that Bart *A* has become infective. A serological test showed that Bart *A*·2 did not react with antiserum prepared from the *A* strain (*A* 121111), indicating that the new strain has not recovered the antigenic properties lost by the *A* strain, but has gained something new, most likely from strain 226. Investigations into the nutritional requirements of the involved strains showed that strain 226 and Bart *A*·2 are greatly stimulated by pantothenic acid, but that Bart *A* is not.

The most likely explanation of the above results is that a transformation induced by the polysaccharide preparation (which might contain DNA) has taken place. This evidence and the earlier report[4] point to the surface material of the *Rhizobium* cells as playing an important part in their ability to infect the root hairs of the appropriate host.

Detailed work on the importance of the capsular material for the infection process is in progress and will be published elsewhere.

I wish to express my indebtedness to Dr. P. S. Nutman for the opportunity to work at his laboratory. My stay at Rothamsted was financed by a grant from Magnus Bergvalls Stiftelse.

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1 Krasilnikov, N. A., *C.R. Acad. Sci., U.R.S.S.*, **31**, 75 (1941).
2 Balassa, R., *Acta Mikrobiologica* (Hungary), **2**, 51 (1954).
3 Balassa, R., *Nature*, **188**, 246 (1960).
4 Ljunggren, H., and Fåhraeus, G., *Nature*, **184**, 1578 (1959).
5 Nutman, P. S., *J. Bact.*, **51**, 411 (1946).
6 Nutman, P. S., *Heredity*, **8**, 35 (1954).
7 Wiley, B. B., and Scherp, H. W., *Canad. J. Microbiol.*, **4**, 505 (1958).
8 Dorn, M., *Zbl. Bact.*, II Abt. **109**, 120 (1956).
9 Sevag, M. G., *Biochem. Z.*, **273**, 419 (1934).

VIROLOGY

Infectious Ribonucleic Acids derived from Mouse Brains infected with Two Kinds of Arbor Virus Group B

Infectious ribonucleic acid (RNA) has been extracted by phenol treatment[1] from cells infected with animal viruses. In Group *B* of the arbor viruses, it has been recently reported that the RNA extracted from mouse brain infected with Murrary Valley encephalitis virus[2] or Japanese *B* virus[3] and from Ehrlich ascites tumour cells infected with West Nile encephalitis[4] are infectious. The purpose of this present communication is to add a datum concerning the extraction of infectious RNA from mouse brains infected with dengue fever virus to the reports of viral RNA already published, and to compare the effects of cold- and hot-phenol extractions on the yields of infectious RNA from the cells infected with Japanese *B* encephalitis virus and dengue fever virus, respectively.

Five-day-old mouse brains infected intracerebrally with the Hawaiian strain of dengue fever virus (*M*-184) and three-week-old mouse brains infected with the Nakayama strain of Japanese *B* encephalitis virus (*M*-55) were employed as the sources of the materials for extraction of RNA. The brains were removed from exsanguinated mice shortly after the appearance of signs of involvement of the central nervous system, ground in a chilled mortar, and a 10 per cent suspension was made with sterile distilled water. Coarse particles were removed by low centrifugation, and the supernatant fluid was used for the experiment. The supernatant fluids were extracted three times with an equal volume of cold 80 per cent phenol at 4° C. for 3 min. following the procedure of Gierer and Schramm[1], or with an equal volume of molten phenol (50° C.) and shaken at 50° C. for 3 min. by Wecker's method[5]. After three such extractions the aqueous phase was shaken five times with peroxide-free ether and the excess ether was removed by bubbling with nitrogen. The aqueous phase obtained by each extraction was reconstituted to the original volume with sterile distilled water. Infectivity titres of the samples, expressed as LD_{50}, were determined by the intracerebral inoculation of serial 10-fold dilutions in mice, 0·02 ml. for three- to five-day-old mice and 0·03 ml. for three-week-old mice. Clarified 10 per cent infected brain suspension and the RNA preparation were regarded as 10^{-1} dilution. The results obtained are shown in Table 1.

Both infectious RNA of Japanese *B* encephalitis virus and dengue fever virus could be easily extracted by phenol treatment, and the yields of infectious RNA of these viruses were the same as that of poliovirus, that is, they amounted usually to about 0·1–0·01 per cent of the crude virus preparation. There were no differences between the effects of cold- and hot-phenol extraction on the yields of RNA derived from the two viruses.

The ultra-violet absorption spectra of these preparations of RNA were typical for nucleic acid. If such an extracted preparation was treated with 50 μgm./ml. of ribonuclease for 10 min. at room temperature prior to inoculation, its infectivity was completely abolished, whereas identical treatment of the virus suspension had no such effect. The preparations of RNA of these viruses had no hæmagglutinating activity under the conditions which were used for these viruses.

Table 1. Infectivity of Two Kinds of Arbor Virus Group *B* and Ribonucleic Acid Preparations

Material	Animal for titration (−log LD_{50}/ml.)	
	Suckling mouse	Adult mouse
Japanese *B* encephalitis virus:		
Crude virus	8·9	8·5
RNA extracted by cold phenol	4·7	4·2
RNA extracted by hot phenol	5·5	3·8
Virus + RNase 50 μgm./ml., 22° C.; 10 min.	9·2	7·5
RNA* + RNase 50 μgm./ml., 22° C.; 10 min.	—†	—
Dengue fever virus:		
Crude virus	8·1	6·0
RNA extracted by cold phenol	4·7	4·5
RNA extracted by hot phenol	4·9	5·0
Virus + RNase 50 μgm./ml., 14° C.; 10 min.	8·0	*N.T.*
RNA* + RNase 50 μgm./ml., 14° C.,; 10 min.	—	*N.T.*

* RNA was extracted by hot-phenol treatment.
† No mice died when given undiluted inoculum.
N.T. = Not tested.

It might be noted, as indicated in Table 1, that, in the case of dengue fever virus, there were no differences between the infectivity titres of RNA for suckling mice and that for adult mice; whereas if adult mice were used for titration, the titres of the crude virus were approximately 100 times lower than that in suckling mice. Therefore, the ratio of virus to RNA in adult mice was much nearer unity than that in suckling mice.

Experiments are now being carried out in the extraction of RNA from adult mouse brains infected with dengue fever virus and suckling mouse brains infected with adult mouse-adapted dengue fever virus.

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[1] Gierer, A., and Schramm, G., *Z. Naturforsch.*, **11**, *b*, 138 (1956).
[2] Ada, G. L., and Anderson, S. G., *Nature*, **183**, 799 (1959).
[3] Nojima, T., *Report from Virus Lab., Kyoto Univ.*, **1**, 101 (1958).
[4] Colter, J. S., Bird, H. H., Moyer, A. W., and Brown, R. A., *Virology*, **4**, 522 (1957).
[5] Wecker, E., *Virology*, **7**, 241 (1959).

Relationship of Tobacco Mosaic Virus (TMV) Lesion Number and Concentration to the Rate of Lesion Production on Pinto Bean

In experiments which required the daily counting of TMV lesions on pinto bean leaves (*Phaseolus vulgaris* L.) for the purpose of determining specific rates of lesion-appearance, it was noted that those leaves which had the greatest final number of lesions produced a greater percentage of their lesions earlier. Since comparisons of rates of lesion development could be seriously affected by differences arising from this, an attempt was made to determine its cause and extent.

Bald[1] had observed the same phenomenon when potato virus *X* was inoculated into *Nicotiana tabacum* L. (var. White Burley), but could not obtain these results when this virus or TMV was inoculated into *N. glutinosa* L. He suggested that either adjacent incipient lesions enhanced production of early lesions or else the greater the number of infective particles entering an infection site, the more rapid the rate of virus multiplication and hence lesion appearance. The first suggestion is not compatible with our results and the second seems unlikely, since it is generally held that only one virus particle can infect one site[2,3]

According to Lauffer and Price[3] the rate of development of symptoms should be a function of concentration of virus, since Bryan and Beard[4] had observed this to be true for rabbit papilloma virus. They postulated that high virus concentrations should be associated with a high probability that every susceptible spot in a scarified area would become infected. Our results with TMV tend to confirm this hypothesis.

In order to investigate this problem more thoroughly a series of concentrations of purified TMV in distilled water, 7, 129, 430, 1,290 and 12,900 μgm./ml., was inoculated to primary pinto bean leaves selected randomly from 10-day-old plants. Inoculation was accomplished by rubbing several drops of virus solution gently with a glass rod over the 600 grit 'Carborundum' sprinkled leaf surface. 1 hr. after inoculation, leaves were washed in running tap water and incubated in a constant light and temperature chamber at 30° C. Lesions were counted at intervals, until new lesions failed to appear. The percentage of the final total of new lesions appearing at each count was plotted against time after inoculation (Fig. 1).

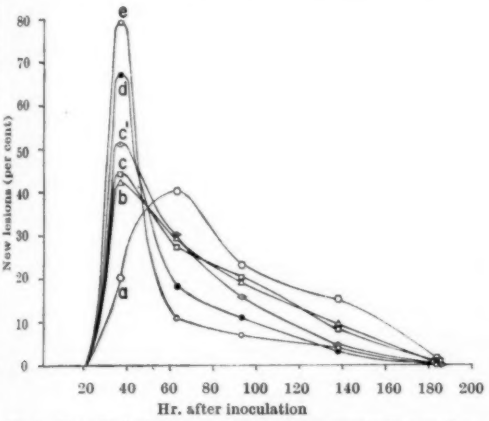

Fig. 1. The effect of tobacco mosaic virus concentration on the rate of appearance of lesions on primary leaves of pinto bean. Virus concentration, number of half-leaves and mean number of lesions per half-leaf for each curve: (*a*) 7 μgm./ml., 6, 9; (*b*) 129 μgm./ml., 8, 48; (*c*) 430 μgm./ml., 8, 61; (*c'*) 430 μgm./ml., 4 of the 8 half-leaves of (*c*) with the greatest number of lesions, 82; (*d*) 1,290 μgm./ml., 6, 60; (*e*) 12,900 μgm./ml., 6, 78. Note that for each time the number of new lesions appearing in the graph is expressed as the percentage of the final total

Examination of the count 37 hr. after inoculation (Fig. 1) shows conclusively that the percentage of new lesions is a function of the virus concentration. It is clear that higher concentrations bring about a higher percentage of lesions appearing early and a lower percentage of those appearing late than do lower virus concentrations. It appears, too, that the difference in the initial rate of appearance of lesions becomes less pronounced as the concentration increases.

Acceleration in appearance of lesions by higher concentrations of TMV was also obtained in two other experiments; one, comparing 0·5 μgm./ml. with 50 μgm./ml. TMV on 18 pinto bean leaves where the average number of lesions per half-leaf for the low concentration was 10 and for the high concentration 62, and the other comparing TMV at 5 μgm./ml. with 50 μgm./ml. on 6 pinto bean leaves where the mean number of lesions per half-leaf for the low concentration was 38 and for the high concentration 93.

An additional observation is that, even though the average number of lesions per half-leaf produced by the 430 μgm./ml. and 1,290 μgm./ml. inocula is about the same, a much greater percentage of the lesions appeared earlier in the case of the more highly concentrated inoculum, as can be seen by comparing the 37 hr. counts (Fig. 1, *d*, *c*). On the other hand, *c'* in Fig. 1, which represents the four half-leaves of the 430 μgm./ml. inoculated group with the most lesions, shows an increased rate of development of lesions as compared with this group as a whole (Fig. 1, *c*, *c'*). Additional comparisons of leaves with high and low lesions counts in four separate experiments averaging fourteen leaves each have confirmed this finding. From calculations[3] based on the number of mixed infections obtained from TMV and aucuba mosaic virus on *N. langsdorffii* Weinm., it seems reasonable to assume that a number of sites within the many-celled area that comprises a lesion by the time it is seen are often exposed and infected in the process of mechanical inoculation. It would follow also that the probability of virus particles initiating an infection at these sites increases with the concentration of the inoculum. Such composite lesions would be likely to appear sooner. The virus concentration is, however, only one variable.

Noting the range of time over which new lesions form for the relatively dilute 7 μgm./ml. inoculum (Fig. 1, *a*), where the percentage of composite lesions should be at a minimum, it appears that sites must vary considerably in the time required for them to produce virus and finally a visible lesion. If such a variability in sites exists, it is possible that at higher concentrations, when more than one site within a lesion area is infected, the site which produces virus most rapidly will determine the time of appearance of the lesion. The time of appearance, then, would depend on the probability of an early site being infected and would be a function of the total number of interactions of virus sites. It is clear that this latter condition depends not only on virus concentration but also on variability in host susceptibility and inoculation technique as well.

This leads us to our finding that when a variety of lesion counts is obtained from a given inoculum, the leaves having the greatest final number of lesions have also the highest percentage of early appearing lesions. Here again, differences in inoculation and host susceptibility have increased the number of interactions of virus sites, resulting in both a higher number of lesions and a higher number of susceptible sites occupied within a lesion area. This results in more and earlier lesions.

It seems apparent, then, that the number of lesions is not what determines the rate of appearance of lesions, but is only a reflexion of the infectivity of the inoculum, inoculating technique and host susceptibility, all of which actually affect the rate. Number of lesions can, however, be used as an index of the number of virus-site interactions.

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[1] Bald, J. G., *Ann. App. Biol.*, **24**, 33 (1937).
[2] Wildman, S. G., *The Viruses*, edit. by Burnet, F. M., and Stanley, W. H., **2**, 1 (Academic Press, New York, 1959).
[3] Lauffer, M. A., and Price, W. C., *Arch. Biochem.*, **8**, 449 (1945).
[4] Bryan, W. R., and Beard, J. W., *J. Infectious Diseases*, **66**, 245 (1940).

Chromatography of Tobacco Mosaic Virus (TMV) on Chitin Columns

While performing experiments on infected tobacco plant fibre to determine the virus-releasing enzymic activity of crude mushroom extracts (Townsley, P. M., unpublished results), it was noted that added chitin strongly adsorbed tobacco mosaic virus. At least a portion of the adsorbed virus could be released with concentrated phosphate buffers. This investigation describes the chromatographic properties of chitin for TMV.

90 gm. of chitin (Eastman Organic Chemicals) was reduced to a fine powder in a porcelain–'Carborundum' ball mill, in about 18 hr. The powder was suspended in 1 litre of *N* sodium hydroxide, stirred for about 0·5 hr. and collected in a Buchner funnel. The chitin pad was washed thoroughly with distilled water and then resuspended in 1 litre of 2 *N* hydrochloric acid. After settling for at least 0·5 hr. the top milky layer was decanted and discarded. The sediment was collected on a Buchner funnel, washed first with water, then with 95 per cent alcohol, and finally with ether.

10 gm. of prepared chitin was suspended in 250 ml. of water, and allowed to settle for 20 min. The top layer was discarded. The sediment slurry, now containing approximately 5 gm. of air-dried chitin, was poured into a chromatographic column, to form a column 17 × 1·3 cm. The column was washed with 25 ml. of 1 *N* hydrochloric acid, followed by 50 ml. of water, then 150 ml. of 0·1 *M tris*–hydrochloric acid, *p*H 6·8, and finally equilibrated with approximately 75 ml. of 0·01 *M tris*–hydrochloric acid, *p*H 6·8 (similar equilibration of the column with phosphate buffer, *p*H 6·8, prevented the column from adsorbing all the added TMV). The eluate from the column was approximately *p*H 6·8.

0·5 ml. of tobacco mosaic virus solution containing 0·3–2 mgm. of virus in 0·01 *M* sodium phosphate buffer, *p*H 6·8, prepared by two alternate cycles at 6,000*g* and 78,000*g* (ref. 1), was added to the column and the virus washed into the column with three successive 1-ml. aliquots of 0·01 *M tris*–hydrochloric acid, *p*H 6·8. 3 ml. of the latter buffer was then placed on the column and the column connected for linear gradient elution. The linear gradient progressed from water to 0·5 *M* K_2HPO_4. The eluate was collected in 4·3-ml. aliquots at 10-min. intervals in a fraction collector equipped for continuous optical density recording at 254 mμ.

As measured by optical density the virus was almost wholly accounted for in the two eluted peaks (Fig. 1). The results were reproducible, but the amount of virus in each peak varied. No explanation is offered for the appearance of two major peaks rather than one as might have been expected from the virus purified by ultra-centrifugation.

Material from both the peaks was birefringent and infective. Preliminary, comparative infectivity tests directly on elution, using six half-leaves of the local lesion host, *Nicotiana glutinosa* L., for each of the peaks, indicated that the material eluted at *p*H 7·2 was from four to ten times as infective as that eluted at *p*H 7·7. Spectrophotometric measurements gave a 260/280 mμ ratio of 1·23 and 1·29 respectively, for the peaks in the eluting solvent.

As a chromatographic agent for TMV chitin would appear to be very useful in view of the mild treatment required for adsorption and elution of the virus.

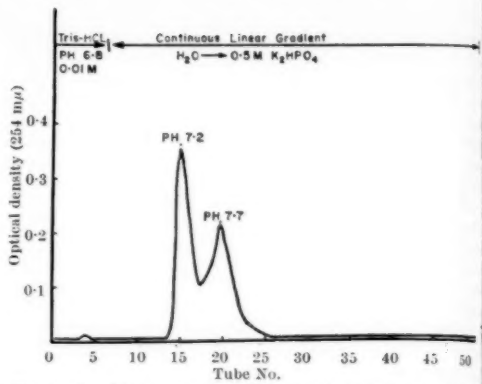

Fig. 1. An elution curve of tobacco mosaic virus from a chitin chromatographic column

Preliminary experiments have also shown that chitin may be used with similar ease for nucleic acid chromatography.

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CYTOLOGY

Peripheral Position of Sex Chromatin

In examining buccal smears for sex chromatin one finds a surprisingly high incidence of sex chromatin bodies not merely at the nuclear membrane but also more precisely at the periphery of what appears to be a disk-shaped nucleus. The following discussion will provide evidence that, as in tissue cultures[1], the sex chromatin is characteristically at the edge of the nuclear disk in squamous cells *in vivo*. In establishing this let us first consider that squamous nuclei *in vivo* are flattened. This may be seen in mucosal smears when a mucosal fragment is folded over (Fig. 1). It is evident, too, that the cells in fragments in the smear maintain the same relative position to one another that they had *in vivo*, and that one is seeing the cells as one would have seen them in looking directly down on to the mucosal surface. Further, in most nuclei which can be reliably scored,

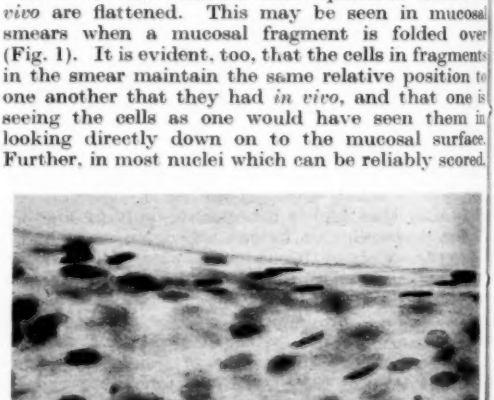

Fig. 1

one can visualize all, or almost all, the nucleoplasm by focusing up and down. Thus one can visualize the sex chromatin regardless of its position in the nucleus.

What is the probability that the sex chromatin body would, by chance alone, appear at the periphery of nuclei as they lie in the optical plane ? Let us assume that the nucleus as observed is an approximate ellipse. If the sex chromatin body lies within a zone bounded by the edge of the nucleus and a locus of points no greater than the thickness of the sex chromocentre measured from the outer to the inner edge (that is, this distance is the altitude when the sex chromatin body is triangular, the radius if the chromatin is plano-convex, and so on), then it will appear to be at the edge of the nucleus. The probability that the sex chromatin will be at the periphery is found by dividing the area of the peripheral zone by the total area of the nuclear ellipse. (Calculations based on volumes are more complicated but give results of the same order of magnitude.)

Ten positive nuclei were selected at random, photographed, and the appropriate dimensions determined. The average thickness of the sex chromatin was slightly less than 0·6μ, while the nuclei averaged about 7·2 × 9·4μ. 0·6μ is smaller than the average figure reported by Prince, Graham and Barr[2] for the short axis of the sex chromatin body, hence in order to weigh the assumptions against the inference being favoured here, the larger figure will be used. The small area in question, that is, the numerator of the probability fraction, is found to a satisfactory approximation by multiplying 0·8 by the circumference of the ellipse. If the width of nucleus is $2a$, the length is $2b$, then the numerator is $0{\cdot}8 \times 2\pi\sqrt{\{(a^2 + b^2)/2\}} = 21\mu^2$; the denominator equals the area of the nuclear ellipse, $\pi ab = 54\mu^2$. Hence the probability that the sex chromatin will lie at the periphery of the nuclear disk equals 21/54 or, approximately, $P = 0{\cdot}39$; that is, about 39 per cent of the positive nuclei might be expected to show sex chromatin at the periphery by chance alone.

Good thionin-stained[3] buccal smears from ten subjects were used in determining the actual incidence of peripheral sex chromatin. One hundred nuclei were counted as being negative, unscorable, or as having peripheral or non-peripheral sex chromatin bodies. Nuclei were listed as unscorable if the observer could not decide whether they were positive or negative because the nuclei were pyknotic, folded over, disrupted or the like. They were scored as having non-peripheral sex chromatin if they had a non-peripheral chromocentre comparable in size (but not necessarily in morphology) to the sex chromatin. Counts were made by me (observer *A*), and by another microscopist (observer *B*). The latter had no knowledge of the purpose of the counts. On the average for both observers, about 86 per cent of the positive nuclei showed the sex chromatin at the periphery. Generally higher counts of observer *A* could properly be attributed to greater experience with sex chromatin assay, but unconscious bias cannot be ruled out.

Since observer *B* found a somewhat smaller percentage of peripheral chromocentres than did observer *A*, observer *B*'s data are used in a test of significance. Observer *B* counted 252 positive nuclei of which 206 had peripheral chromatin bodies, while in 46 there were non-peripheral bodies. Using the theoretical expectation of 39 per cent peripheral chromocentres gives corresponding figures of 98 peripheral and 154 non-peripheral. $\chi^2 = 199{\cdot}5$, $P < 0{\cdot}005$. This evidence favours the view that the sex chromatin is characteristically at the periphery of the nuclear disk in smears, and since the relative orientation and shape of the nuclei are similar, the same observation applies to cells *in vivo*.

In addition to conditions in tissue cultures[1] mentioned here, preferential localization of sex chromatin has been described also for the cigar-shaped nuclei of smooth muscle cells[4], where the chromatin tended to be at one tip or the other. The mechanisms responsible for these localizations remain to be elucidated.

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[1] Miles, C. P., *Cancer*, **12**, 299 (1959).
[2] Prince, R. H., Graham, M. A., and Barr, M. L., *Anat. Rec.*, **122**, 153 (1955).
[3] Klinger, H. P., and Ludwig, K. S., *Stain Technol.*, **32**, 235 (1957).
[4] Castro, N. M. de, and Sasso, W. da S., *An. Pac. farmacia Odont. S. Paulo*, **11**, 249 (1953). Moore, K. L., and Barr, M. L., *Acta Anat., Basel*, **21**, 197 (1954). Hungerford, D. A., Donnelly, A. J., Nowell, P. C., and Beck, S., *Amer. J. Human Genet.*, **11**, 215 (1959).

Chromosomal Elimination induced by 6-Aza-Uracil

DURING preliminary work in this Laboratory dealing with the effects of some antimetabolites on cell division in *Allium cepa*, it was noted that 6-aza-uracil brings about chromosomal elimination. This elimination occurred at metaphase and anaphase. Root tips from bulbs after reaching a length of 2–3 cm. were treated for 6 hr. with a 1 per cent aqueous solution. The solution was prepared by first grinding the powder and dissolving it in warm tap water. In order to clarify the location of the spindle attachment region of the chromosomes the root tips were treated with a 1 per cent aqueous solution of colchicine for a half-hour prior to fixation. On each preparation the number of normal metaphases, anaphases and telophases and the number of these stages showing elimination were counted. The eliminated chromosomes were studied in detail to determine whether or not the same chromosomes were always eliminated. Aceto-orcein smears and sections stained in iron alum hæmatoxylin were used.

Following 6-hr. treatment with the chemical 13 per cent of the division figures showed eliminated chromosomes. Further examination of mitotic figures indicated that anaphase figures showed this phenomenon more frequently than any other stage with an average of 31·7 per cent of the figures showing elimination. In different roots anaphase elimination ranged from 16 to 54 per cent.

Of 456 metaphases analysed, 6·4 per cent showed eliminated chromosomes. Of these, 66 per cent eliminated one chromosome (Fig. 1*A*), 24 per cent eliminated two chromosomes, 7 per cent eliminated three chromosomes and 3 per cent eliminated six chromosomes. In most of these cells the metaphase chromosomes were clumped and the eliminated

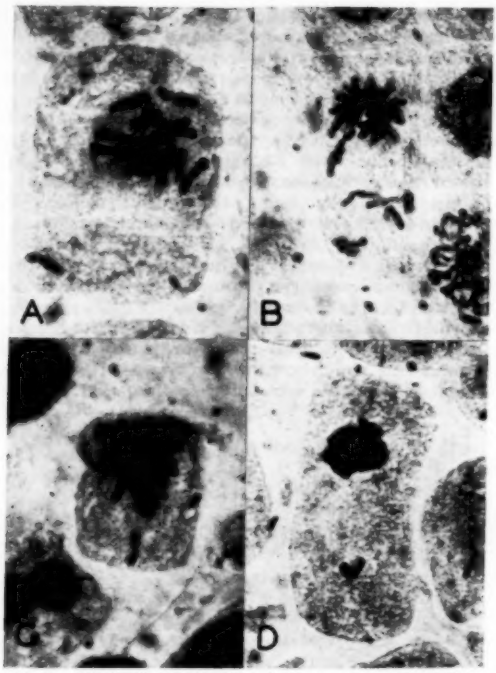

Fig. 1. Photomicrographs of aceto-orcein smear preparations of cells from the root tip of *Allium cepa* showing chromosomal elimination. *A*, metaphase; *B*, anaphase; *C*, anaphase; *D*, telophase. (× 750)

chromosomes were some distance from this mass and near the cell membrane. Sectioned material showed that these divisions with eliminated chromosomes occur in both the plerome and the periblem of the meristematic region. The eliminated chromosome was not always the same. Chromosomes with median, sub-median and sub-terminal centromeres were eliminated. These nuclei were apparently in prometaphase when affected by the chemical.

The anaphase picture is even more aberrant. In some cases two anaphase groups were noted with lagging chromosomes. It is assumed that such cells were in anaphase when influenced by the chemical. In other cases one large anaphase group was present with a number of chromosomes scattered throughout the cell (Fig. 1*B*). A similar effect was noted by Deysson[1] in *Allium sativum* following treatment with sodium oxalate and sodium citrate. In still other cases one large anaphase group was present with a single eliminated chromosome near the periphery of the cell (Fig. 1*C*). In other cases there appears to be no orientation at all. Two hundred and fifty-two anaphase figures were analysed. Of these 31·7 per cent showed eliminated chromosomes. Of these cells showing elimination 35 per cent eliminated one chromosome, 15 per cent eliminated two chromosomes, 16·3 per cent eliminated three chromosomes, 10 per cent eliminated four chromosomes, 7·5 per cent eliminated five or six chromosomes, 3·7 per cent eliminated eight chromosomes and 2·5 per cent eliminated nine or ten chromosomes. The types of chromosomes were eliminated at random.

At telophase in most cases there was a tightly clumped mass of chromosomes in various stages of reversion with one, two, three or four chromosomes near the periphery of the cell forming micronuclei (Fig. 1*D*). In other cells there were two telophase groups of chromosomes, but with apparently varying chromosome numbers. Even following normal anaphases cell-plate formation is apparently interfered with and binucleate cells frequently result. It is possible that these cells were affected in late anaphase, since anaphase separation itself was not interfered with. In those cells showing chromosomal elimination, the resulti g nuclei are frequently of unequal size. Telophase figures were difficult to analyse. Of 210 cells studied, 11 cells or 5·2 per cent showed eliminated chromosomes. Of the cells 54·5 per cent eliminated one chromosome and 18·2 per cent eliminated two chromosomes.

Several investigations have been undertaken to ascertain the exact mechanism responsible for chromosomal elimination. Geyer-Duszynska[2] proposed that certain cytoplasmic factors inhibit chromosomal elimination, but she did not suggest any mechanism for the initiation of elimination of chromosomes. However, Avanzi[3] threw some light on the subject by showing that thallium acetate inhibits the functioning of the centromere in some chromosomes which afterwards lag at anaphase. Brachet[4] and Janaki-Ammal[5] also suggested a defective centromere as an explanation for chromosomal elimination. Nicklas[6,7] stated that elimination of chromosomes was due to failure of normal chromosomal fibre action. Vaarama[8] presented evidence that split spindles and timing disturbances resulted in the uneven distribution of chromosomes at the end of metaphase and his diagrams show the failure of some chromosomes to be included in the spindle structure.

Elimination of chromosomes by treatment with 6-aza-uracil may find a possible explanation in the fact that this substance is an analogue of uracil which is a normal constituent of ribonucleic acid. Davies[9] showed that in situations where the normal nucleic acid metabolism of the cell has been upset, the centromeres behave abnormally. He stated that a close interrelationship exists between the centromeric activity, nucleic acid metabolism and chromosomal behaviour. Evidence that ribonucleic acid is needed for the functioning of the spindle was reported by Kaufmann and Das[10], who showed that after treatment with ribonuclease, mitotic abnormalities were found, such as contraction, stickiness, adhesion and clumping of chromosomes, production of aneuploid and polyploid chromosome complexes, tripolar and multipolar spindles, binucleate cells and lagging of chromosomes at anaphase.

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[1] Deysson, G., *Pathologie et Biologie*, Tenth Congr. Intern. Biol. Cell., **9**, 500 (1961).
[2] Geyer-Duszynska, I., *Bull. Acad. Polon. Sci.*, **6**, 371 (1958).
[3] Avanzi, S., *Caryologia*, **9**, 131 (1956).
[4] Brachet, J., *Arch. Biol.*, **65**, 1 (1954).
[5] Janaki-Ammal, E. K., *Nature*, **146**, 839 (1940).
[6] Nicklas, D. B., *Chromosoma*, **10**, 301 (1959).
[7] Nicklas, R. B., *Chromosoma*, **11**, 402 (1960).
[8] Vaarama, A., *Hereditas*, **35**, 136 (1949).
[9] Davies, D. R., *Chromosoma*, **8**, 221 (1956).
[10] Kaufmann, B. P., and Das, N. K., *Chromosoma*, **7**, 19 (1955).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

TUTORIAL STUDENT (graduate or about to graduate) IN GEOGRAPHY to read for a higher degree and give a limited amount of assistance by demonstrating—The Registrar, King's College (University of London), Strand, London, W.C.2 (August 9).

LECTURER (with a good honours degree, and teaching and/or research experience) TO TEACH PHYSICS to General Degree and G.C.E. Advanced level; and Grade B ASSISTANTS (2) (preferably with teaching and/or research experience) TO TEACH (*a*) PHYSICS and (*b*) MATHEMATICS—The Principal, Norwood Technical College, Knight's Hill, London, S.E.27, quoting Ref. FE.3a/N/1926/7 (August 12).

RESEARCH ASSISTANT (with a first- or second-class honours degree in physics, and prepared to register for a higher degree) IN THE DEPARTMENT OF PHYSICS for work in the field of electron spin resonance—The Registrar, The University, Nottingham (August 12).

LECTURER or ASSISTANT LECTURER (with a good honours degree in electrical engineering or physics, with special qualifications in one of the following branches: electrical machines, plasmas, materials, high vacuum or solid state electronics) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING—The Secretary, The University, Edinburgh (August 14).

LECTURER (with a specialist interest in and experience of climatology and meteorology) IN GEOGRAPHY—The Registrar, University College, Singleton Park, Swansea (August 14).

ASSISTANT (with a special interest in pig husbandry) IN ANIMAL HUSBANDRY—The Secretary, The University, Aberdeen (August 19).

DEMONSTRATORS IN THE DEPARTMENT OF GEOLOGY—The Registrar, University College of North Staffordshire, Keele, Staffs (August 19).

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RESEARCH ASSISTANT (honours graduate in biochemistry or microbiology, or closely related fields with subsidiary biochemistry) IN MICROBIOLOGICAL BIOCHEMISTRY to carry out research on problems of the regulation of metabolism in bacteria—The Secretary of University Court, The University, Glasgow (August 19).

SENIOR LECTURER (with outstanding qualifications and achievements in a relevant field of electrical engineering or physics) IN ELECTROMAGNETISM—The Assistant Registrar (Science), The University, Edgbaston, Birmingham 15 (August 19).

ELECTRONICS DESIGNER (with a degree in physics or electrical engineering, or a comparable qualification) IN THE DEPARTMENT OF CHEMISTRY to collaborate with members of the teaching staff in designing and constructing electronic equipment to be used in research work in the Department—The Registrar, The University, Manchester 13 (August 21).

PRINCIPAL OF THANET TECHNICAL COLLEGE—The Clerk to the Governors, Education Office, Denemount, The Vale, Broadstairs (August 22).

LECTURER IN AFRICAN ANTHROPOLOGY—The Secretary, School of Oriental and African Studies, University of London, London, W.C.1 (August 31).

RESEARCH ASSISTANT IN THE DEPARTMENT OF CIVIL ENGINEERING, to work with Dr. J. M. Zarek on problems of flow measurements under pulsating conditions—The Registrar, King's College, Strand, London, W.C.2 (August 31).

SENIOR LECTURER IN THE DEPARTMENT OF BIOCHEMISTRY (Sub-Department of Malting and Brewing)—The Assistant Registrar (Science), The University, Edgbaston, Birmingham 15 (August 31).

UNIVERSITY LIBRARIAN; and CHAIRS OF AGRICULTURE, ARCHÆOLOGY, EDUCATION, ZOOLOGY, BOTANY, CHEMISTRY, PHYSICS, BIOCHEMISTRY, MATHEMATICS, GEOGRAPHY, PHARMACY, CIVIL ENGINEERING and SOCIOLOGY in the University of Ife, Nigeria—The Registrar, University of Ife (Ibadan Branch), Ibadan, Nigeria (August 31).

DIRECTOR OF RESEARCH—Prof. A. C. Frazer, British Food Manufacturing Industries Research Association, Randalls Road, Leatherhead, Surrey (September 1).

ASSISTANT IN ZOOLOGY IN THE DEPARTMENT OF NATURAL HISTORY—The Secretary, The University, Aberdeen (September 2).

RESEARCH ASSISTANT (preferably male, with an honours degree in social anthropology or psychology) IN THE DEPARTMENT OF SOCIAL ANTHROPOLOGY, to conduct an industrial study in the Midlands—The Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh (September 4).

SENIOR LECTURER (with a higher degree or equivalent qualifications and interested in promoting and leading research) IN MATHEMATICS in the Broken Hill Division of the University of New South Wales—The Agent General for New South Wales, 56–57 Strand, London, W.C.2, and the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (September 8).

RESEARCH ASSOCIATE (graduate in psychology) IN THE DEPARTMENT OF SOCIAL AND ADMINISTRATIVE STUDIES, UNIVERSITY OF OXFORD, for a project, sponsored by the National Science Foundation, Washington, concerned with perception—The Secretary, Barnett House, 40 Wellington Square, Oxford (September 10).

LECTURER or ASSISTANT LECTURER IN AGRICULTURAL ZOOLOGY at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (September 15).

SENIOR LECTURER or LECTURER IN PHYSICS at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 15).

TEACHING FELLOW (with an honours degree or equivalent qualifications) IN THE SCHOOL OF MATHEMATICS, University of New South Wales, Australia—The Agent General for New South Wales, 56–57 Strand, London, W.C.2, and The Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (September 19).

CHAIR OF VETERINARY PHYSIOLOGY—The Secretary to the University, The University, Edinburgh (October 1).

ANALYST (preferably B.Sc. (Chemistry), and G.R.I.C., or equivalent qualifications) IN THE DEPARTMENT OF ORGANIC CHEMISTRY, for duties mainly concerned with the operation and care of optical equipment including infra-red and nuclear magnetic resonance spectrometers—Mr. W. A. Wightman, School of Chemistry, The University, Leeds 2.

ASSISTANT EXPERIMENTAL OFFICER (with a pass degree in biology (including zoology), F.I.M.L.T. or equivalent qualifications, and preferably some experience of histological techniques) IN THE PHYSIOLOGY DEPARTMENT to assist with histological investigations related to the endocrine research programme of the Department—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 61/22.

FORESTRY OFFICER (with an honours degree in forestry and a sound practical approach and ability to train and guide supervisory staff, preferably with experience in tropical forestry), to deal with all aspects of forestry work in Hong Kong, particularly with the preparation of management plans and in-service training—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/51/07/T.

GEOLOGIST (with at least a first- or upper second-class honours degree in geology, and preferably specialized in petrology) with the Falkland Islands Dependencies Survey for service in the Antarctic for two years—The Crown Agents, 4 Millbank, London, S.W.1, quoting Ref. M3C/53437/NF.

GRADUATE to participate in biochemical research on coagulation in normal human and human cancer tissue—Dr. W. A. Boggust, Department of Biochemistry, Trinity College, Dublin.

GRADUATE (with a degree in botany or zoology, and preferably previous experience in cytological work) for work in a section investigating cytogenetic effects of radiation—The Administrator, Radiobiological Research Unit, Medical Research Council, Harwell, Didcot, Berks.

LECTURER or ASSISTANT LECTURER IN PHYSICS at Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

LECTURER or ASSISTANT LECTURER (particularly interested in petrology and mineralogy) IN GEOLOGY—Prof. D. Williams, Geology Department, Imperial College of Science and Technology, London, S.W.7.

LECTURER (with special qualifications in pure mathematics) IN MATHEMATICS (Grade I or II)—The Registrar (Room 22, O.R.B.), The University, Reading.

LIBRARIAN to take charge of a technical library—The Director of Research, The Natural Rubber Producers' Research Association, 48–56 Tewin Road, Welwyn Garden City, Herts.

MICROBIOLOGIST or BIOCHEMIST (recent graduate) for one year's sponsored research on antibiotics—Dr. J. G. David, 9 Gerrard Street, London, W.1.

RESEARCH ASSISTANT (preferably with experience in cytology), to take part in a programme designed to study human chromosome abnormalities and associated disorders—The Secretary, Genetics Department, The University, Sheffield 10.

RESEARCH ASSISTANT to take part in chromosome studies on human leukæmias and other forms of malignant disease using tissue culture techniques—The Administrative Officer, Mount Vernon Hospital, Northwood, Middlesex.

RESEARCH FELLOW (with Ph.D. degree or equivalent research experience) IN THE CHEMISTRY DEPARTMENT, to work in collaboration with Dr. J. J. Kipling on the carbonization and graphitization of high polymers—The Professor of Chemistry, The University, Hull.

SENIOR RESEARCH ASSOCIATES (2) (preferably experienced electrical engineers or physicists with an electrical engineering background) IN THE DEPARTMENT OF PHYSICS for the development of the proton synchrotron—The Assistant Registrar (Science), The University, Edgbaston, Birmingham, 15.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The National Foundation for Educational Research in England and Wales. *Technical Education Abstracts from British Sources*, Vol. 1, No. 1 (September–December, 1960.) Pp. 57+xxvii. Subscription rate: 42*s.* per volume of four quarterly issues. (London: National Foundation for Educational Research in England and Wales, 1961.) [305

British Museum (Natural History). The Culicine Mosquitoes of the Indomalayan Area. Part 5: Genus *Aëdes* Meigen, subgenera *Mucidus* Theobald, *Ochlerotatus* Lynch Arribalzaga and *Neomelaniconion* Newstead. By P. F. Mattingley. Pp. 62. (London: British Museum (Natural History), 1961.) 15*s.* [305

University of Reading: National Institute for Research in Dairying. Report, 1960. Pp. 164. (Shinfield: University of Reading: National Institute for Research in Dairying, 1961.) [305

Chelsea College of Science and Technology. Prospectus, Session 1961–62. Pp. 35. (London: Chelsea College of Science and Technology, 1961.) [305

Ministry of Housing and Local Government. Pollution of water by Tipped Refuse: Report of the Technical Committee on the Experimental Disposal of House Refuse in Wet and Dry Pits. Pp. 141+7 plates. (London: H.M. Stationery Office, 1961.) 7*s.* net. [315

Machine Tool Industry Research Association. News Letter, No. 1 (May, 1961). Pp. 19. (Manchester: Machine Tool Industry Research Association, 1961.) [315

Production Engineering Research Association of Great Britain. PERA Report No. 50: Drilling Carbon Steels. Pp. 21. (Melton Mowbray: Production Engineering Research Association of Great Britain, 1961.) [315

University of Reading: Museum of English Rural Life. Report, 1960. Pp. 19 (5 plates). (Reading: The University, 1961.) 1*s.* [16

National Society for Clean Air. Clean Air Year Book, 1961. Pp. 80. (London: National Society for Clean Air, 1961.) 2*s.* 6*d.* net. [16

C.I.B.A. (A.R.L.), Limited. Technical Notes, No. 221 (May, 1961): Aerolite in Scotland. Pp. 12. (Duxford: C.I.B.A. (A.R.L.), Ltd., 1961.) [16

Bulletin of the British Museum (Natural History). Zoology. Vol. 7, No. 7: The Dealfishes (Trachipteridae) of the Mediterranean and North-East Atlantic. By G. Palmer. Pp. 335–351+plate 62. (London: British Museum (Natural History), 1961.) 8s. [26

Committee of the Privy Council for Agricultural Research. Report of the Agricultural Research Council for the year 1959–60. Pp. ix+209. (Cmnd. 1362). (London: H.M. Stationery Office, 1961.) 9s. net. [26

The University of Leeds. Publications and Titles of Theses, 1958–9. Pp. 79. (Leeds: The University, 1961.) 2s. 6d. [26

Oundle School Natural History Society. Report for 1960. Pp. ii+45. (Oundle, Peterborough: Oundle School Natural History Society, 1961.) [26

A Study Outline. Prepared by Brian Groombridge. Pp. 57. (London: National Union of Teachers, 1961.) 1s. 6d. [66

The Imperial Forestry Institute, University of Oxford. Thirty-sixth Annual Report, 1959–60. Pp. 32. (Oxford: Imperial Forestry Institute, The University, 1961.) [66

Department of Scientific and Industrial Research: Forest Products Research. Special Report No. 15: Working Stresses for Structural Laminated Timber. By W. T. Curry. Pp. v+50+4 plates. (London: H.M. Stationery Office, 1961.) 4s. net. [66

National Union of Teachers. Some Thoughts on Higher Education: a speech delivered by Sir Wilfred Martineau at the Annual Conference, Brighton, 1961. Pp. 12. Sixth Form Studies: an address by H. D. P. Lee to the Grammar Schools Meeting at the Annual Conference, Brighton, 1961. Pp. 16. Looking Ahead: an address by Sir Ronald Gould, General Secretary of the N.U.T. to the Union's Annual Conference at Brighton, 1961. Pp. 16. (London: National Union of Teachers, 1961.) [66

Department of Scientific and Industrial Research: Road Research Laboratory. Road Research Technical Paper No. 52: The Use and Control of Vegetation on Roads and Airfields Overseas. By K. E. Clare. Pp. vi+42+8 plates. (London: H.M. Stationery Office, 1961.) 3s. 6d. net. [66

Public Investment and Export. By Sir Walter Benton Jones. Bart. Pp. 7. (Grantham: Sir Walter Benton Jones, Irnham Hall, 1961.) [66

International Nickel Company (Mond), Ltd. Nickel in S.S. *Canberra*. Pp. ii+22. (London: International Nickel Company (Mond), Ltd., 1961.) [66

Department of Scientific and Industrial Research. Hydraulics Research 1960: The Report of the Hydraulics Research Board with the Report of the Director of Hydraulics Research. Pp. iv+79+8 plates. (London: H.M. Stationery Office, 1961.) 5s. 6d. net. [76

Building Research Station Digest (Second Series). No. 11: Heating in Local-Authority Flats. Pp. 4. (London: H.M. Stationery Office, 1961.) 4d. [76

Other Countries

Bulletins de la Société Royal de Zoologie d'Anvers. No. 15: Les Kiwis (Apterygiformes dans les Systemes de Classification. Par Prof. R. Verheyen. Pp. 11. No. 16: Liste des Mammiferes ayant figuré ou figurant dans les collections du Jardin Zoologique de la Société Royale de Zoologie d'Anvers, depuis sa fondation en 1843 jusju au mois de Juin 1960 (inclus), avec én regard l'année la plus reculée au cours de laquelle leur présence fut établie. Données réunies par Dr. Agatha Gijzen. Pp. 18. No. 17: Contribution à l'Osteologie et à la Systematique des Ratitae. Par Dr. R. Verheyen. Pp. 16. No. 18: *Anomalurus jacksoni* de Winton: Répartition, Biologie et Observations en Captivité. Par U. Rahm. Pp. 16 (5 planches). No. 19: Les Poissons d'Aquarium du Bassin du Congo. Par J. G. Lambert. Pp. 50 (27 planches). No. 20: Observations sur la Natation chez les Reptiles. Par R. R. Tercafs. Pp. 19 (6 planches). (Anvers: Société Royale de Zoologie d'Anvers, 1960 et 1961.) [305

Académie Royal de Belgique. Annuaire pour 1961. Pp. 320+7 planches. (Bruxelles: Académie Royale de Belgique, 1961.) [305

Kirkia, Vol. 1, 1960–1961. Edited by H. Wild. (Journal of the Federal Herbarium, Salisbury, Rhodesia and Nyasaland.) Pp. 164+20 plates. (Salisbury: Government Printer, 1961.) 30s. [305

Uganda Electricity Board. Thirteenth Annual Report and Accounts for the year ended 31st December, 1960. Pp. 48. (Kampala: Uganda Electricity Board, 1961.) 5s. [305

Food and Agriculture Organization of the United Nations. Animal Health Branch Monograph No. 3: A Laboratory Handbook of Veterinary Diagnostic Procedures. By A. J. Stevens. Pp. ii+70. (Rome: Food and Agriculture Organization of the United Nations, 1961.) [305

Annals of the New York Academy of Sciences, Vol. 92, Article 1: Vitamin C. By J. J. Burns, C. G. King and 53 other authors. Pp. 1–332. (New York: New York Academy of Sciences, 1961.) [305

Metropolitan Life Insurance Company. Statistical Bulletin, Vol. 42 (April, 1961): Rapid Growth in Populous Areas. Death Claim Payments Exceed $568,000,000 in 1960. Accident Fatalities in Canada. Tuberculosis is Being Wiped Out. Pp. 12. (New York: Metropolitan Life Insurance Company, 1961.) [315

United States Department of Agriculture: Agricultural Research Service. Technical Bulletin No. 1227: A Revision of the Species of *Monopsyllus* Kolenati in North America (Siphonaptera, Ceratophyllidae. By Phyllis T. Johnson. Pp. ii+69 (22 plates). (Washington, D.C.: Government Printing Office, 1961.) 35 cents. [315

United States Department of the Interior: Geological Survey. Water-Supply Paper 1535–C: Calculation and Use of Ion Activity. By John D. Hem. Pp. iii+17+1 plate. (Washington, D.C.: Government Printing Office, 1961.) [315

International Centre for the Study of the Preservation and the Restoration of Cultural Property, Rome. Works and Publications, III: Climacology and Conservations in Museums. Pp. 201–289. (Reprinted from *Museum*, Vol. XIII/No. 4, 1960.) (Rome: International Centre for the Study of the Preservation and the Restoration of Cultural Property, Via Cavour 256, 1960.) [315

Bulletin of the Bingham Oceanographic Collection, Peabody Museum of Natural History, Yale University. Vol. 17, Article 4: Studies on Spawning Behaviour, Egg Masses, and Larval Development in the Gastropod Genus *Conus*. 2: Observations in the Indian Ocean during the Yale Seychelles Expedition. By Alan J. Kohn. Pp. 51. (New Haven, Conn.: Peabody Museum of Natural History, Yale University, 1961.) 2 dollars.

Institut Royal Meteorologique de Belgique. Observations Ionospheriques—Station de Dourbes. Février, 1960. Pp. 26. Mai 1960. Pp. 26. Publications, Série A, No. 16: La Station d'Aerologie de l'Institut Royal Meteorologique de Belgique. Par P. Defrise, Maenhout et J. Van Mieghem. Pp. 24. (Bruxelles: Institut Royal Meteorologique de Belgique, 1961.)

Fact Sheets on U.S. Nuclear Power Projects. Third edition. Pp. iii+51. (New York: Bozell and Jacobs, Inc., 230 Park Avenue, 1961.)

Food and Agriculture Organization of the United Nations. Report of the FAO/WHO Seminar on Problems of Food and Nutrition in Africa South of the Sahara, Lwiro, Bukavu (Congo), 18–29 May, 1959 (FAO Nutrition Meetings Report Series, No. 25.) Pp. ix+93. (Rome: Food and Agriculture Organization of the United Nations; London: H.M. Stationery Office, 1961.) 5s.; 1 dollar.

National Academy of Sciences—National Research Council. Nuclear Science Series. NAS–NS 3022: The Radiochemistry of Vanadium. By J. L. Brownlee, Jr. Pp. v+76. 75 cents. NAS–NS 3029: The Radiochemistry of Ruthenium. By E. I. Wyatt and R. R. Rickard. Pp. vii+78. 1 dollar. (Washington, D.C.: Office of Technical Services, Department of Commerce, 1960 and 1961.)

United States Department of Commerce: Coast and Geodetic Survey. Publication 65–1, Part 19: Plane Coordinate Intersection Tables (2½ minute), Massachusetts. Pp. iii+27. 25 cents. Technical Bulletin No. 16 (January, 1961): Erosion and Sedimentation, Eastern Chesapeake Bay at the Choptank River. By G. F. Jordan. Pp. ii+8+1 map. 40 cents. (Washington, D.C.: Government Printing Office, 1961.)

Legislative Council of Fiji. Council Paper No. 5 of 1961: Geological Survey Department—Annual Report for the year 1960. Pp. ii+10. (Suva: Geological Survey Department, 1961.) 1s. 6d.

Chicago Natural History Museum. Fieldiana: Geology. Vol. 14, No. 5 (April 26, 1961): Eurypterids of the Devonian Holland Quarry Shale of Ohio. By Erik N. Kjellesvig-Waering. Pp. 79–98. 85 cents. Fieldiana: Zoology. Vol. 41, No. 3, (April 2, 1961): New Caledonian Land and Fresh-Water Snails: an Annotated Check List. By Alan Solem. Pp. 413–501. 2.50 dollars. Vol. 42, No. 9 (April 7, 1961): Philippine Zoological Expedition, 1946–1947. A New Genus and Species of Cantacaderina Lace-Bug from the Philippines. (Hemiptera: Tingidae). By Carl J. Drake. Pp. 115–118. 20 cents. (Chicago: Chicago Natural History Museum, 1961.)

United States Department of the Interior: Geological Survey. Professional Paper 303–D: Geology of the Shaviovik and Sagavanirktok Rivers Region, Alaska: Exploration of Naval Petroleum Reserve No. 4 and Adjacent Areas, Northern Alaska. Part 3: Areal Geology. By A. Samuel Keller, Robert H. Morris and Robert L. Detterman. Pp. iii+169–222+plates 21–26. (Washington, D.C.: Government Printing Office, 1961.)

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Paper 60–31: Belleville and Wellington Map-Areas, Ontario, (30 N/14 and part of 30 N/15 W½: 31 C/3 and part of 31 C/2 W½). By B. A. Liberty. Pp. ii+9+2 maps. 50 cents. Geological Maps—Preliminary Series. Map 41–1960: Big Bald Mountain, Northumberland County, New Brunswick. Map 43–1960: Mingo Lake, Baffin Island, District of Franklin, Northwest Territories. Map 48–1960: St. Mary Bay, Digby, Yarmouth and Charlotte Counties, Nova Scotia and New Brunswick. Map 50–1960: North Spirit Lake, Kenora District, Ontario. Map 52–1960: Whiskey Jack Lake, Manitoba. Map 1–1961: Hopewell, (West Half), Nova Scotia. (Ottawa: Queen's Printer, 1960 and 1961.)

Annals of the South African Museum. Vol. 46, Part 1: Cercopithecoid Fossils, including a New Species, from Taung, Cape Province, South Africa. By Leonard Freedman. Pp. i–14+plates 1–10. 36c. Vol. 46, Part 2: Some New Caddis Flies (Trichoptera) from the Western Cape Province, III. By Dr. K. M. F. Scott. Pp. 15–54. 33c. Vol. 46, Part 3: Some South African *Cladocera* collected by Dr. A. D. Harrison. Pp. 35–46. 21c. Vol. 46, Part 4: Pogonophora from South Africa. By Eve C. Southward. Pp. 47–53. 13c. (Cape Town: South African Museum, 1961.)

Companhia de Diamantes de Angola (DIAMANG). Servico Culturais. Dundo—Lunda—Angola. Museu do Dundo. Publicacoes Culturais No. 52: Subsidios para o Estudo da Biologia na Lunda–Estudos Diversos (XX). Pp. 271. (Lisboa: Companhia de Diamantes de Angola, 1961.)

United States of America. Scientific Progress, The University and the Federal Government: Statement by the President's Science Advisory Committee. Pp. v+33. (Washington, D.C.: Government Printing Office, 1960.) 15 cents.

Indian Forest Records (New Series.) Statistical—1957. Vol. 1, No. 1: A Statistical Study of the Variability of Physical and Mechanical Properties of *Tectona grandis* (Teak) Grown at Different Localities of India and Burma and the Effects of the Variability on the Choice of the Sampling Plan. By K. R. Nair and H. K. Mukerji. Pp. 1+49. Rs. 8.00.; 13s. Silviculture. Vol. 10, No. 9: Standard Volume Tables for *Dipterocarpus macrocarpus* Vesque. By S. K. Seth and S. N. Dabral. Pp. 239–246. Rs. 1.55.; 2s. 6d. (Delhi: Manager of Publications, 1960.)

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